Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 39, No. 6, June 1982

Volume 39, n° 6, juin 1982

Canada

Fisheries and Oceans / Pêches et Océans

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

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Volume 39, No. 6, June 1982

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 39, No. 6, June 1982 Volume 39, n° 6, juin 1982

Relative Effectiveness of Two Ichthyoplankton Sampling Techniques[1]

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CADA, G. F., AND J. M. LOAR. 1982. Relative effectiveness of two ichthyoplankton sampling techniques. Can. J. Fish. Aquat. Sci. 39: 811–814.

Two common ichthyoplankton sampling devices, a pump and a conical plankton net, were evaluated for their relative effectiveness in collecting various sizes of clupeid larvae in the headwaters of Watts Bar Reservoir in eastern Tennessee. Triplicate surface pump and towed-net samples were taken during both day and night on three dates in June 1979 to detect any diel differences in sampler effectiveness. Clupeid larvae, 4–10 mm long, were collected in numbers large enough to make meaningful statistical comparisons of the two techniques. The differences in the mean densities of most length groups between nighttime pump and tow samples were not statistically significant. During the day, however, the pump collected significantly more 4-mm larvae and the towed net collected more 5- to 10-mm larvae. Our results indicate that under conditions by which these samplers are commonly deployed in open waters, most sizes of young shad larvae exhibited a greater ability to avoid the pump sampler than the towed net during the daytime.

Key words: ichthyoplankton, pump, nets, fish larvae, avoidance

CADA, G. F., AND J. M. LOAR. 1982. Relative effectiveness of two ichthyoplankton sampling techniques. Can. J. Fish. Aquat. Sci. 39: 811–814.

L'article qui suit contient une évaluation de deux appareils communément utilisés dans l'échantillonnage de l'ichtyoplancton, la pompe et le filet à plancton conique, par rapport à leur efficacité relative à capturer des larves de clupéidés de diverses tailles à la source du réservoir de Watts Bar, dans l'est du Tennessee. Les échantillons ont été prélevés en triplicata en surface, avec pompe et filet à plancton, le jour et la nuit, à trois dates en juin 1979. Le but était de détecter des différences nycthémérales d'efficacité des échantillonneurs. Des larves de clupéidés de 4 à 10 mm de long ont été capturées en nombres suffisants pour permettre des comparaisons statistiques significatives entre les deux méthodes. Les différences de densité moyenne de la plupart des groupes de longueur des échantillons nocturnes de la pompe et du filet ne sont pas significatives à l'analyse statistique. Par contre, de jour, la pompe capture nettement plus de larves de 4 mm, tandis que le filet en capture davantage de 5 à 10 mm. Nos résultats indiquent que, dans les conditions dans lesquelles ces appareils

[1]Publication No. 1922, Environmental Sciences Division, ORNL.

Printed in Canada (J6558)
Imprimé au Canada (J6558)

sont ordinairement utilisés en pleine eau, les jeunes aloses de presque toutes les tailles sont plus aptes à éviter la pompe que le filet traîné durant le jour.

Received June 26, 1981
Accepted February 10, 1982

Reçu le 26 juin 1981
Accepté le 10 février 1982

ACCURATE estimates of ichthyoplankton densities and size structure are important for a variety of fisheries applications, ranging from year-to-year comparisons of production to the assessment of environmental stresses (e.g. power plant entrainment) on fish populations. The two most commonly used types of ichthyoplankton field sampling gear are conical plankton nets and pump samplers (Bowles and Merriner 1978). Nets have the capability of sampling a large volume of water in a short time, but they are susceptible to clogging and the attendant loss of filtration efficiency. Pumps, on the other hand, eliminate the problem of mesh clogging and permit the sampling of more precise volumes and locations, but they can be hampered by biases associated with relatively small sample volumes. Both techniques are subject to some degree of sampler avoidance by motile organisms and extrusion through the meshes of the collecting net.

Although many ichthyoplankton field studies utilized towed-net or pump sampling techniques (reviews by Bowles and Merriner 1978; Carpenter 1979), critical evaluations of the comparability of these gear types with regard to such factors as avoidance or extrusion have rarely been reported. Further, gear evaluations are frequently based on comparisons of total densities of selected taxa (Aron 1958; Gale and Mohr 1978; Leithiser and Davidson 1981), with no consideration given to differential behavior by various sizes of fish within a taxon. It is generally assumed that for a given species sampling gear avoidance is directly related to size. Significant differences between samplers in avoidance by larger larvae may be masked by nonsignificant differences among smaller, more-abundant larvae. In research efforts which require an accurate description of the size and age structure of the ichthyoplankton community (e.g. population modeling), size-specific avoidance could be an important source of error.

This study was designed to examine the relative effectiveness of pump and towed-net samplers, as they are commonly deployed in open waters, in collecting various sizes of clupeid larvae. In particular, size-specific effectiveness of the two gear types was evaluated by comparing estimated densities of each length group in a series of diel samples.

Materials and Methods

Clupeid larvae were collected at two sampling stations in upper Watts Bar Reservoir (Clinch River) in eastern Tennessee. Based on adult fish studies, the larvae were probably of the genus *Dorosoma*, although positive identification was not possible. One station was located in the main channel of the Clinch River at river kilometre 18.5. Water depth at this station was 10 m. The second sampling station was located in Poplar Creek, a tributary of the Clinch River, ~0.8 km upstream from its mouth at Clinch River kilometre 19.3. Depth of the water ranged from 3 to 4 m. A detailed description of the study area is provided by Loar (1981).

Ichthyoplankton tow samples were collected at the surface (0–0.5-m depth) by towing a 2-m-long, 0.5-m-mouth-diameter Hensen egg net (equipped with a nonporous mouth-reducing cone) ~18 m behind the boat. The net was composed of 0.243-mm mesh Nitex and had an open area ratio (R) of 1.79. Sample volumes and towing velocities were determined by means of an impeller-type flowmeter (General Oceanics number 2030) mounted in the center of the mouth of the net. The net was towed for 5 min at velocities of 120–189 cm/s (average, 145 cm/s), generally resulting in sample volumes of between 85 and 100 m^3.

Pump samples were collected by means of a Sears Homelite trash pump adjusted to provide a flow rate of 1.1 m^3/min. The 7.6-cm-diameter intake hose was fitted with a cylindrical trash screen with 6.5-cm^2-square openings. The pump intake was placed 0.25 m below the surface off the bow of the boat, which was slowly moved through the water to avoid sampling the same area continuously. Pump discharge water was filtered through the same net used for tow samples, which was held suspended in water at the side of the boat by a specially constructed brace. Each sample consisted of a 15-min pumping period, resulting in a sample volume of 16.7 m^3. The intake velocity through the openings of the trash screen was ~110 cm/s.

Sampling was conducted during three diel periods (June 13–14, June 21–22, and June 28–29, 1979) at the time of peak larval abundance (Loar 1981). Triplicate daytime samples were collected between 09:50 and 16:40 for each sampling method, station and diel period and comparable triplicate nighttime samples were taken on the following evening between 22:50 and 05:45. To minimize disturbance of the sampling area with the boat's prop wash, we collected the triplicate pump samples before the triplicate tow samples.

Samples were field preserved in 5% Formalin solution. All larvae were identified to the lowest possible taxonomic level, counted, and, to a maximum of 100 randomly selected individuals per sample, measured for length. The total number of larvae in each length class per sample was extrapolated from the relative proportion of that length class among fish actually measured.

We used analyses of variance on log-transformed data to detect significant differences in clupeid densities due to the effects of sampling stations, dates, techniques, times of day (day vs. night), and fish lengths. The significance of differences among means for significant main effects were tested using Duncan's Multiple-Range Test.

Results

Both sampling techniques collected clupeid larvae ranging from 4 to 17 mm in total length, although larvae greater than 10 mm long were caught in relatively small numbers. The incidence of collection damage was low in both pump and net samples and was not biased toward any particular size-class. Analysis of variance tests on total densities indicated that all main effects (date, station, time of day, length, and sampling technique) were significant sources of variability at

TABLE 1. Comparisons of clupeid larval densities in diel pump and towed-net samples.

Total length (mm)	Total no. in day tow samples	Mean density in day tow samples (no./m³)	Total no. in day pump samples	Mean density in day pump samples (no./m³)	P^a	Total no. in night tow samples	Mean density in night tow samples (no./m³)	Total no. in night pump samples	Mean density in night pump samples (no./m³)	P
4	78	0.04	60	0.18	0.003	350	0.17	63	0.17	0.847
5	20 423	2.93	1604	1.28	<0.001	1374	0.55	215	0.52	0.716
6	3 417	1.21	229	0.40	0.017	158	0.09	19	0.06	0.211
7	879	0.37	7	0.02	<0.001	48	0.03	17	0.05	0.101
8	318	0.16	6	0.02	<0.001	43	0.02	12	0.04	0.468
9	46	0.03	0	0.00	0.003	6	<0.01	11	0.04	0.002
10	47	0.03	0	0.00	0.008	12	0.01	9	0.03	0.004
11	8	<0.01	0	0.00	0.327	7	<0.01	6	0.02	0.029
12	9	<0.01	0	0.00	0.255	8	<0.01	3	<0.01	0.544
13	0	0.00	0	0.00	—	7	<0.01	6	0.02	0.070

[a]Probability of observing a larger value of F, under the null hypothesis that the mean densities in tow and pump samples are equal.

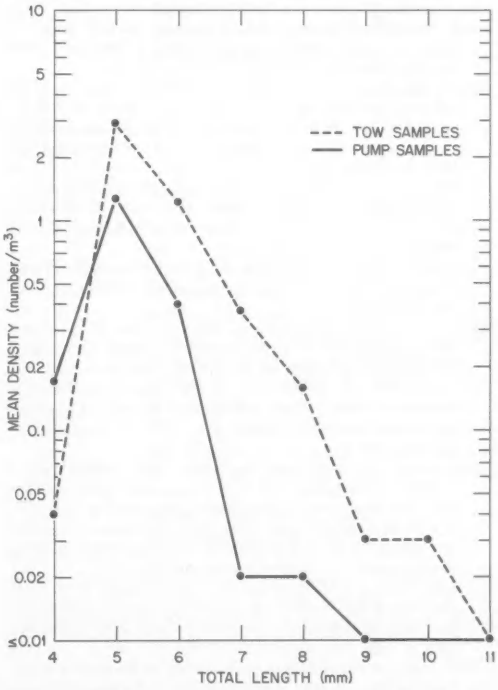

FIG. 1. Mean densities of various lengths of clupeid larvae in daytime pump and tow samples.

the $\alpha = 0.01$ level. In addition, several of the interaction effects, notably the sampling technique times time-of-day interaction, were statistically significant.

Further analyses of variance were conducted to compare the densities of clupeid larvae (averaged over all dates and stations) collected by the two techniques as a function of fish length and time of day (Table 1). The mean densities of most length groups between nighttime pump and towed-net samples were not significantly different ($P > 0.05$). During the day, however, the pump collected significantly more 4-mm larvae and the towed net collected more 5- to 10-mm larvae (Fig. 1). Although some statistically significant differences between sampling techniques were noted among larvae ≥9 mm long in nighttime samples, these differences are probably not meaningful in view of the small numbers of these larvae that were collected (Table 1). A total of 13 larvae, 14–17 mm in length, were collected, and in no case was the difference between pump and tow sample densities significant at the $\alpha = 0.05$ level.

Discussion

As deployed in this study, the towed net was more effective than a low volume pump at collecting most sizes of early clupeid larvae during the day. Although the size range over which we observed these differences was small (5–10 mm total length), they are the most abundant sizes and accounted for 98% of all clupeid larvae collected in this study. Because differential extrusion was not a factor (the same net was used in both pump and towed samples) and because the towed net had a higher entrance velocity than the pump, we attribute our observations to greater avoidance of the pump intake.

The fact that significant differences between pump and towed net collections were not observed during the night sampling suggests that the stimulus for greater pump avoidance was visual rather than tactile, e.g. response to physical disturbances ahead of the sampler. However, it should be noted that mean densities of clupeid larvae in both types of samples were lower at night than during the day. Concurrent sampling in deeper strata indicated that clupeid larvae ≥5 mm total length exhibited diel vertical migrations, occurring in higher densities at the surface during the day and at greater depths (3–4 m) during the night. It is possible that nonvisual avoidance was responsible for gear differences observed in the daytime surface samples, and insufficient numbers of larvae were collected to demonstrate that same avoidance mechanism at night.

Vertical stratification of ichthyoplankton can complicate

gear comparisons if the sampling devices are operated at different depths. For example, one sampler may be deployed in deeper, more plankton-rich waters than another, and the resulting differences in densities could mistakenly be interpreted as avoidance or extrusion. Pump intakes withdraw water from all directions rather than from a narrow stratum, and when simultaneously drawn through the water would trace out a roughly cylindrical path similar to that of a towed net. This consideration, coupled with mixing within a riverine system, makes it unlikely that vertical microdistribution of clupeid larvae could have affected our comparisons.

Based solely on a consideration of sampler intake velocities and the resultant potential for avoidance, it would generally be expected that pumps are more effective than fixed nets, that towed nets are more effective than low volume pumps, and that high volume pumps are equally or more effective than towed nets in collecting ichthyoplankton. These patterns have been demonstrated in some comparative studies (Leithiser et al. 1979; Leithiser and Davidson 1981; Zeitoun et al. 1981; present study), but there are also a number of apparent exceptions (Portner and Rohde 1977; Gale and Mohr 1978; King et al. 1981). Portner and Rohde (1977) speculated that their unexpected results may have been due to some bias in the calibration or operation of one of the collection systems. Sampler avoidance in the studies by Gale and Mohr (1978) and King et al. (1981) may not have been detected because of a preponderance of prolarvae and eggs, respectively, and relatively small numbers of larger larvae in many of the samples. Further, King et al. (1981) considered pump damage to be a potential factor in their comparisons. Thus, it is important in gear evaluations to report not only the intake velocities and relevant sampling information, but also the incidence of collection damage and the size range of ichthyoplankton over which reliable comparisons may be made.

In our study, total densities of clupeid larvae reflected the same pattern that emerged from consideration of particular size-classes, i.e. that the towed net was more effective than the pump. However, if only total densities were considered, comparison of the samplers under conditions in which relatively more 4-mm-long larvae were present, e.g. on an earlier date or closer to the upstream spawning sites, could have led to the erroneous conclusion that the samplers were equally effective or that the pump was more effective. Any gear comparison should take into account size selectivity and the potential for smaller, more-abundant size-classes to mask true differences between the samplers. This is particularly important in impact assessment studies, such as entrainment surveys, where the loss of larger larvae, and therefore need for good density estimates, may be more serious.

No ichthyoplankton sampling technique is completely effective in collecting all sizes of fish larvae. As a result, the sampler will display a bias, characteristic of the mode of deployment and sampling conditions, which must be controlled or quantitatively accounted for to obtain an accurate picture of the ichthyoplankton community. Earlier studies showed that under specific situations (e.g. a power plant cooling water intake canal), a high-volume pump may be preferable to a fixed plankton net, whose effectiveness is constrained by low current velocities. Even in these cases, however, care should be exercised because a pump may not adequately sample all entrainable organisms. That is, an organism moving passively with the cooling water flow might still avoid the abrupt currents associated with a pump intake. Although low volume pump samplers may be necessary in certain field situations where towed or push nets or large, high-volume pumps cannot be used (e.g. intake canals, weedy or shallow areas), our research suggests that serious consideration must be given to the potential of relatively small, weakly swimming fish larvae to avoid the pump intake.

Acknowledgments

We express our appreciation to Jean A. Solomon for her assistance in the statistical analyses. We thank Drs Lawrence W. Barnthouse and D. S. Vaughan of the Oak Ridge National Laboratory (ORNL) and Lee F. Graser of the Tennessee Valley Authority for their reviews of the manuscript. Research was sponsored by Ecological Research Division, Office of Health and Environmental Research, U.S. Department of Energy, under contract W-7405-eng-26 with Union Carbide Corporation.

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Species Replacement in a Reservoir Fish Community: Silverside Feeding Mechanics and Competition

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MCCOMAS, S. R., AND R. W. DRENNER. 1982. Species replacement in a reservoir fish community: silverside feeding mechanisms and competition. Can. J. Fish. Aquat. Sci. 39: 815–821.

The brook silverside (*Labidesthes sicculus*) was the only atherinid species found in Lake Texoma, Oklahoma, from 1944 to 1953. Inland silversides (*Menidia beryllina*) were first collected in Lake Texoma in 1953 and had completely replaced *Labidesthes* by 1955. We conducted comparative studies of feeding mechanics and feeding efficiencies of *Labidesthes* and *Menidia* to see if there was a competitive differential between species that could have been a factor in the replacement of *Labidesthes* by *Menidia*. *Menidia* has a protrusible and tube-shaped mouth that is more successful capturing evasive copepod prey than the *v*-notched mouth of *Labidesthes*. While both species have similar feeding rates for slow-swimming daphnids, *Menidia* has higher feeding rates than *Labidesthes* for cyclopoid and calanoid copepods. *Menidia*'s use of copepod prey is a competitive advantage over *Labidesthes* in laboratory competition experiments. We hypothesize the same advantage was an important factor in displacing *Labidesthes* from Lake Texoma.

Key words: planktivory, *Labidesthes sicculus*, *Menidia beryllina*, Lake Texoma

MCCOMAS, S. R., AND R. W. DRENNER. 1982. Species replacement in a reservoir fish community: silverside feeding mechanisms and competition. Can. J. Fish. Aquat. Sci. 39: 815–821.

Le crayon d'argent (*Labidesthes sicculus*) a été la seule espèce d'athérinidé présente dans le lac Texoma, en Oklahoma, entre 1944 et 1953. Des capucettes (*Menidia beryllina*) ont été capturées pour la première fois dans ce lac en 1953 et, dès 1955, avaient complètement remplacé *Labidesthes*. Nous avons mené des études comparatives des mécanismes d'alimentation et des rendements alimentaires de ces deux espèces afin de vérifier si une compétition différentielle entre espèces aurait pu jouer un rôle dans le remplacement de *Labidesthes* par *Menidia*. Cette dernière a une bouche protractile et tubiforme, pouvant mieux capturer une proie évasive comme les copépodes que la bouche échancrée en *v* de *Labidesthes*. Les deux espèces ont des taux d'alimentation semblable avec daphnies à nage lente. Mais, dans le cas de copépodes cyclopoïdes et calanoïdes, *Menidia* a des taux plus élevés que *Labidesthes*. Dans des expériences de compétition en laboratoire, l'utilisation de copépodes comme proies par *Menidia* lui donne un avantage sur *Labidesthes*. Nous croyons que ce même avantage a été un important facteur dans le déplacement de *Labidesthes* du lac Texoma.

Received August 10, 1981
Accepted March 3, 1982

Reçu le 10 août 1981
Accepté le 3 mars 1982

A fish species replacement occurred during 1954 and 1955 in the planktivore community of Lake Texoma, a 37 600-ha impoundment (in 1944) of the Washita and Red rivers located on the Texas–Oklahoma border. The brook silverside (*Labidesthes sicculus*) was living in tributary streams prior to impoundment (Sisk and Stephens 1964) and was the only silverside in the reservoir until 1953 (Moore and Buck 1953; Dowell and Riggs 1958). The inland silverside (*Menidia beryllina*), formerly known as the Mississippi silverside (*Menidia audens*) (Chernoff et al. 1981), was first discovered in the Red River drainage in 1949 (Moore and Cross 1950) and in Lake Texoma in 1953 (Riggs and Bonn 1959), but was not common in the lake until the summer of 1954. *Labidesthes* populations declined through 1954 (Riggs and Dowell 1956) and by 1955 were found only in Lake Texoma's tributaries (Saunders 1959) or wherever tributaries entered the lake (Riggs and Dowell 1956). Seining collections in Lake Texoma in 1957 and 1958 revealed that *Menidia* was the only silverside inhabiting the reservoir (Dowell and Riggs 1958; Dalquest 1957, 1958; Riggs and Bonn 1959). A reproducing *Labidesthes* population has not been found anywhere in Lake Texoma since 1955, although they still inhabit the tributaries flowing into the reservoir today (Anthony Echelle, Oklahoma State University, OK, personal communication). A similar silverside replacement occurred in the early 1970s in Keystone reservoir, an impoundment of the Arkansas and

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Printed in Canada (J6582)
Imprimé au Canada (J6582)

Cimarron rivers in central Oklahoma, which was filled in 1964 (Sisk and Stephens 1964; Gomez and Lindsay 1972). The causes for *Labidesthes* replacement by *Menidia* in the two reservoirs have not been determined, although interspecific competition has been suggested (Sisk and Stephens 1964).

In lakes, both silverside species are planktivores, occasionally feeding on insects or fish fry (Boesel 1937; Saunders 1959; Mullen et al. 1968; Taylor 1975; Elston and Bachen 1976). Planktivore feeding efficiency is influenced by mouth morphology (O'Brien 1979). Given a similar buccal volume and suction time, a protrusible tube-shaped mouth will have a more powerful suction than a forcep-shaped open mouth. *Labidesthes* and *Menidia* are similar morphologically except for marked differences in mouth structure. We found that adult *Labidesthes* have a forcep-shaped open mouth, while adult *Menidia* have a protrusible tube-shaped mouth which could potentially result in greater capture success for evasive zooplankter prey such as adult copepods (Drenner et al. 1978). We studied feeding mechanics and feeding efficiencies of *Menidia* and *Labidesthes* and conducted laboratory competition experiments to see if a competitive feeding difference existed between these two species, which could have been a factor in *Labidesthes*'s disappearance from Lake Texoma.

Materials

The study was conducted at the University of Oklahoma Biological Station on Lake Texoma. *Menidia* were seined from Lake Texoma and *Labidesthes* were dipnetted out of Carter Lake and City Lake, Marshall County, Oklahoma, which are within 40 km of Lake Texoma. Specimens used in the mouth protrusibility measurements were preserved in 10% Formalin. Mouth protrusibility was computed by subtracting snout length (distance from the anterior edge of the eye orbit to the anterior tip of the tightly closed mouth) from the distance, with mouth completely open, between anterior edge of eye orbit and posterior junction of premaxillae and mandible. Measurements were made using a dissecting microscope and an eyepiece micrometer.

Zooplankton were obtained from Lake Texoma (*Mesocyclops*, *Cyclops*, and *Diaptomus*) and Carter Lake (*Daphnia*, *Ceriodaphnia*, and *Diaphanosoma*). Cladoceran and copepod body length was measured as distance between anterior edge of head to base of tail spine or end of cephalothorax, respectively. Body length was measured by using a dissecting microscope with an eyepiece micrometer.

Experimental Procedure

Capture success for *Labidesthes* and *Menidia* was determined by visual observation of fish feeding on zooplankton prey in aquaria. Five fish of each species 50–65 mm standard length (SL) were put in separate aquaria (29 cm × 30 cm × 46 cm) with diffuse overhead light and black background. Zooplankton densities were ~200 organisms·L^{-1}.

The feeding rates of *Menidia* and *Labidesthes* for zooplankton were determined in 4- to 7.5-h laboratory feeding experiments conducted in 45-L aquaria at 22 ± 2°C under fluorescent laboratory lights with four groups of four to six *Menidia* and four groups of four to six *Labidesthes*. *Labidesthes* ranged from 58 to 79 mm SL ($\bar{x}$SL = 74.0, SD = 5.2). *Menidia* ranged from 55 to 90 mm SL ($\bar{x}$SL = 69.0, SD = 13.2). Silversides used in the feeding experiments were acclimated for at least 24 h. Feeding experiments were run in clear or turbid water. Turbidity was created by adding silt and clay particles collected from the offshore area of Lake Texoma to the aquaria. Turbidity was measured with a DR-EL/1 Hach kit colorimeter in Formizin Turbidity Units (FTU). Clear water turbidity ranged from 0 to 9 FTU. Initial turbid water readings ranged from 150 to 160 FTU and final turbidities ranged from 110 to 120 FTU. During the summers of 1978 and 1979, *Menidia* feeding experiments were conducted in clear (n = 8) and turbid water (n = 7). In 1979, seven *Labidesthes* feeding experiments were conducted in both clear and turbid water.

Feeding experiments were begun by pouring an array of freshly collected zooplankton into an aquarium containing fish. After gently stirring the aquarium, initial zooplankter densities were sampled by lowering a clear Plexiglas tube (5.8 cm ID) onto a rubber stopper randomly placed on the aquarium bottom. Two samples were taken at the beginning and at the conclusion of the experiment. All samples were preserved in 10% Formalin. Initial zooplankter densities varied from 450 to 4000 organisms·L^{-1}. Control aquaria containing no fish did not reveal other causes of zooplankton mortality.

Feeding rate constants were calculated using Dodson's (1975) equation: $k = \ln(P_i/P_f)/XT$, where k = feeding rate constant, P_i = initial zooplankters·L^{-1}, P_f = final zooplankters·L^{-1}, X = fish·L^{-1}, and T = time in hours. Feeding rate per gram of fish body weight (k/g) was computed by dividing feeding rate constant by average body weight of fish.

Laboratory experiments were set up to examine effects of competition between *Labidesthes* and *Menidia* for food. Fish used in the competition experiments were young-of-the-year. *Labidesthes* ranged from 17 to 49 mm SL ($\bar{x}$SL = 32.0, SD = 7.0). *Menidia* ranged from 22 to 50 mm SL ($\bar{x}$SL = 34.0, SD = 8.6). Three treatments were used. Each treatment consisted of an aquarium holding both *Labidesthes* and *Menidia* and control aquaria containing the silverside species separately. To hold intraspecific competition constant, we placed a similar number of individuals of a species in the control and competition aquaria.

In treatment 1, fish received TetraMin flake fish food and freshly captured Lake Texoma zooplankton every 3–4 d. Zooplankton were collected with an 80-μm net. Nine *Labidesthes* or *Menidia* were contained in each 15-L control aquarium and 10 fish of each species were held together in the 15-L competition aquarium.

In treatment 2, all aquaria received only Lake Texoma zooplankton. Because of the high silverside density, sampling zooplankton after their introduction into the aquaria was avoided to reduce the risk of fish mortality. Composition of the zooplankton was determined by subsampling the collected zooplankton prior to their introduction into the aquaria. Initial zooplankton densities in the aquaria were ~1000 organisms·L^{-1}. The zooplankton assemblage averaged 2% daphnids, 31% *Diaphanosoma*, and 67% copepods during the competition experiments. In the first replicate, each 64-L control aquarium contained 18 *Labidesthes* or *Menidia* held separately. The 64-L competition aquarium held 20 *Labidesthes* and 19 *Menidia*. In the second replicate of treat-

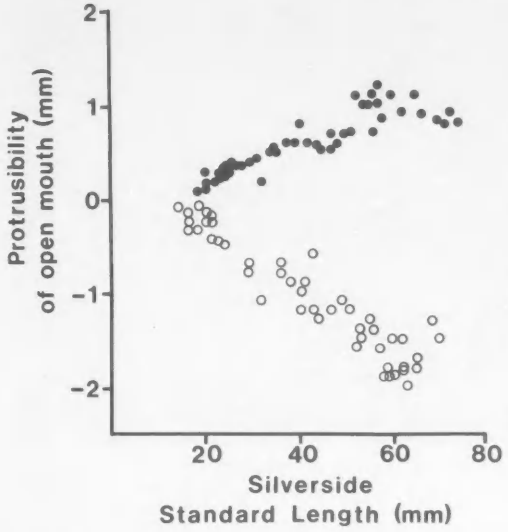

FIG. 1. Mouth protrusibility of *Labidesthes* (open circles) and *Menidia* (solid circles). Positive numbers indicate that the end of the open mouth protrudes or extends past the end of the closed mouth. Negative values indicate that the measurement of the end of the oral branchial chamber is less than the closed mouth snout length.

ment 2, seven *Labidesthes* or nine *Menidia* were held in 22-L control aquaria. The 22-L competition aquarium held seven *Labidesthes* and eight *Menidia* . Treatment 3 provided a baseline starvation rate. No zooplankton or fish food was added to the filtered lake water. Twenty *Labidesthes* or *Menidia* were held separately in the 69-L control aquaria. Twenty individuals of each species were held together in the 69-L competition aquarium.

Results

Feeding Mechanics and Capture Probabilities

The mouths of both *Labidesthes* and *Menidia* smaller than 20 mm SL have circular openings and are not very protrusible (Fig. 1). Mouth morphologies change with maturation of both species. *Menidia*'s protrusibility increases with body size. Lowering of the mandible pulls the maxilla forward, sliding the premaxillae anteriorly over the rostral cartilage and expanding the oral branchial chamber. In contrast, *Labidesthes*' mouth protrusibility decreases as the fish increases in length. When an adult *Labidesthes* opens its mouth, ligaments prevent the ascending process of the premaxilla from sliding over the rostral cartilage. The premaxilla and maxilla of adult *Labidesthes* form an acute angle to the mandible resulting in a forcep-like mouth shape that contrasts with the tube-like mouth of adult *Menidia* (Fig. 2).

The difference in silverside mouth morphologies is associated with differences in approach strategies used to attack zooplankton prey. Under lighted conditions, both silversides are visual feeding planktivores that attack individual prey items. Aquarium observations revealed that *Menidia* initiate an attack from a point 1–2 cm from the prey; cruising *Labidesthes* start an attack 5–6 cm away from a prey by using a strong caudal tail flick that propels the mouth through a prey position. The mouth of both species opens just prior to prey contact. In the final stages of prey capture, *Labidesthes*' recurved teeth may aid in grasping the prey. *Menidia* inhale prey with the incurrent water flow, and they do not require teeth to capture prey. Teeth of *Menidia* in Lake Texoma are greatly reduced relative to *Labidesthes*.

Differences in fish attack and feeding mechanics resulted in different capture probabilities for zooplankton prey. *Menidia*'s short attack coupled with the efficient inhalation mechanics produced a higher capture success for copepods than did the lunging attack of *Labidesthes* (Table 1). Capture success for daphnid prey was ~100% for both fish. We could not observe silverside capture success for nauplii, but nauplii are easily captured by fish. Rosenthal (1969) found herring (*Clupea harengus*) larvae had 100% success in capturing nauplii.

Feeding Experiments

Menidia was size-selective for zooplankton prey. Its feeding rate constants per gram of fish body weight (k/g) increased as a linear function of zooplankter size in clear and turbid water (Fig. 3). The functional relationship of k/g in clear water versus daphnid and nauplii size was best fit by the equation $k/g = 0.46X - 0.05$ ($r^2 = 0.99$) where X is prey length. The function of feeding rate constants in turbid water versus daphnid and nauplii size was best fit by the equation $k/g = 0.45X + 0.06$ ($r^2 = 0.82$).

Menidia feeding rates on copepods were slightly lower than feeding rates on daphnids and nauplii, but *Menidia* was still size-selective for copepods in clear and turbid water ($k/g = 0.31X - 0.04$; $r^2 = 0.99$) and ($k/g = 0.54X - 0.08$; $r^2 = 0.66$), respectively.

Labidesthes was also size-selective for daphnids and nauplii (Fig. 3). Their feeding rate constants increased as a function of zooplankter body size ($k/g = 0.35X$; $r^2 = 0.88$). In turbid water the results were similar and feeding rate constants versus daphnid and nauplii size were best fit by the equation $k/g = 0.31X + 0.05$ ($r^2 = 0.73$). *Labidesthes* had low feeding rates for copepod prey and did not appear size-selective (Fig. 3). Their feeding rate constants as a function of copepod body size were $k/g = 0.04X + 0.03$ ($r^2 = 0.89$) in clear water and $k/g = 0.06X$ ($r^2 = 0.98$) for turbid water.

Differences in feeding mechanics resulted in *Labidesthes* and *Menidia* having different feeding rates for copepods. In clear water, *Menidia* had higher feeding rates than *Labidesthes* for *Diaptomus* (*t*-test: $P < 0.05$) (Fig. 3). In turbid water, *Menidia* feeding rates for all three copepods were significantly higher ($P < 0.05$) than *Labidesthes* feeding rates. Differences in fish-feeding mechanics do not affect fish capture success on zooplankton with poor escape abilities. *Menidia* and *Labidesthes* had similar feeding rates for daphnid and nauplii in both clear and turbid water. Fish-feeding rates on the cladoceran *Diaphanosoma* are shown in Fig. 3, but were not included in the regression analyses because *Diaphanosoma* escape ability is intermediate between daphnids and copepods.

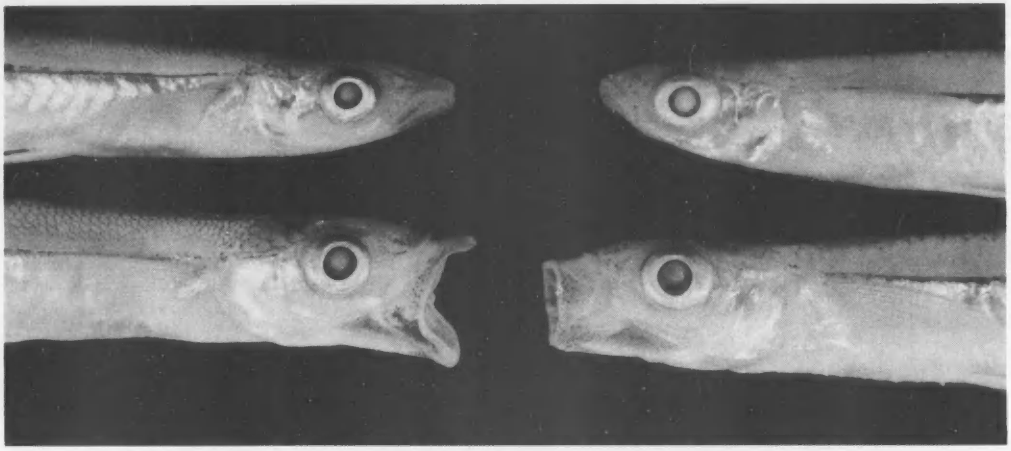

FIG. 2. Differences in open- and closed-mouth morphologies of *Labidesthes* (left) and *Menidia* (right).

COMPETITION EXPERIMENTS

In treatment 1 (Fig. 4-1), Lake Texoma zooplankton and TetraMin fish food were added to the aquaria every 3 or 4 d. Survivorship ranged from 89 to 100% for all combinations of fish, demonstrating that *Menidia* and *Labidesthes* could coexist in an aquarium for at least 25 d with abundant nonevasive food. In treatment 3 (Fig. 4-3), when no food was added to the aquaria, mortality rates in the control were similar to mortality rates in the competition aquaria for both species. At the end of 23 d, mortality was 85–100%.

In treatment 2 (Fig. 4-2a, b), Lake Texoma zooplankton were the only food source. *Labidesthes* mortality increased above the controls when in combination with *Menidia* in both replicates of the competition experiments. After 22 and 30 d, *Labidesthes* experienced 75 and 86% mortality, respectively, when in combination with *Menidia* compared to 25 and 0% mortality in the *Labidesthes* control aquarium. In comparison, *Menidia* experienced 0 and 12% mortality in the same competition experiments and 11 and 22% mortality in the control. The more easily captured zooplankton were quickly eliminated in the competition aquaria, leaving copepods, which constituted two-thirds of the initial zooplankton assemblage, and some *Diaphanosoma*, a zooplankter with intermediate escape ability. The dominant zooplankton observed 3 or 4 d after introduction into the competition aquaria were copepods that were not used by *Labidesthes* as a sustaining food source.

Discussion

Interspecific competition occurs when one species' use of a limited resource reduces the resource available to another species. The intensity and eventual outcome of interspecific competition is a function of the niche overlap between species (May and MacArthur 1972; Pianka 1974; Diamond 1978; but see Kohn 1971). Niche overlap between species has been characterized by analyzing similarities in habitat and food habits (Orians and Horn 1969; Zaret and Rand 1971; Werner and Hall 1976). Field studies and seining records indicate

TABLE 1. Percent capture success on zooplankter prey. Number of attempts is in parentheses.

	Menidia	*Labidesthes*
Ceriodaphnia and *Daphnia*	98 (200)	100 (15)
Diaphanosoma	78 (23)	50 (10)
Calanoid copepods	73 (59)	40 (25)
Cyclopoid copepods	53 (171)	39 (59)

that *Labidesthes* and *Menidia* use similar habitats in Lake Texoma. As suggested by Cahn (1927) and Saunders (1959), our shoreline seining has indicated that both fish are nearshore from dusk to dawn and move into the pelagic zone during the day. The two species do not partition the pelagic zone by depth; both species remain in the upper few metres (Hubbs 1921; Cahn 1927; Mense 1967; Hubbs et al. 1971).

The food habits of *Labidesthes* and *Menidia* also overlap. Both species are particulate feeders under lighted conditions, feeding selectively on the largest prey they can capture. The primary diet difference is that *Menidia* can capture copepods more efficiently than *Labidesthes*. Field studies show that *Labidesthes* and *Menidia* feed on prey types that would be predicted from their feeding mechanics. The most common zooplankter prey in young *Labidesthes* diets in early summer are daphnids (Keast 1965; Keast and Webb 1966; Mullen et al. 1968; Taylor 1975). As *Labidesthes* mature, they supplement their diet with an increasingly high proportion of surface-caught chironomids or other dipterans (Zimmerman 1970; Taylor 1975), which would have low escape abilities. Adult chironomids are generally not found in young *Labidesthes*, possibly because the insects are too large to be ingested. Although *Labidesthes* feed on copepods, they rarely constitute a major food source for *Labidesthes* (Keast 1965; Keast and Webb 1966; Mullen et al. 1968; Taylor 1975). In contrast, *Menidia*'s stomachs not only contain daphnids and chironomids (Saunders 1959; Elston and Bachen 1976), but copepods can make up a dominant part of the diet. Saunders (1959) found up to 89% of the prey items of *Menidia* were copepods.

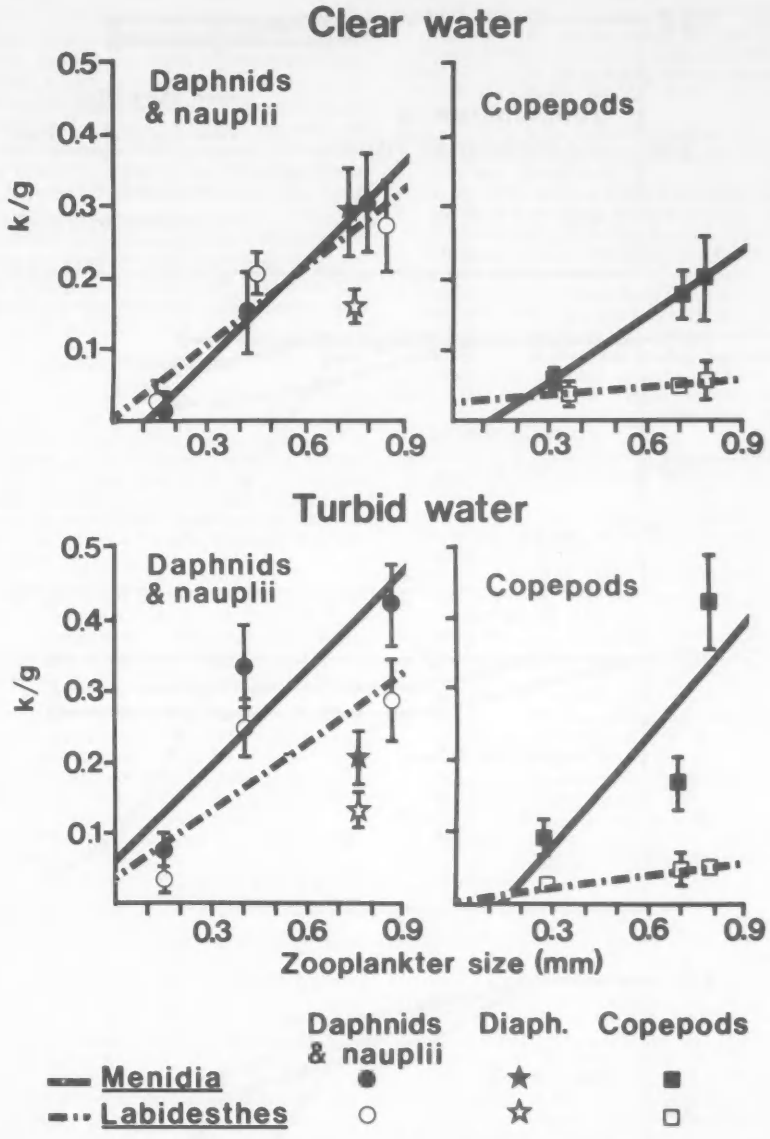

FIG. 3. Silverside feeding experiments. Means ± SE of fish-feeding rates, experienced as a constant k per gram of fish body weight, are split into categories for the zooplankter array. Easily caught prey include *Daphnia* (largest), *Ceriodaphnia*, and nauplii (smallest). Evasive prey include the copepods *Mesocyclops* (largest), *Diaptomus*, and *Cyclops* (smallest). *Diaphanosoma* is a cladoceran having intermediate escape ability and is in a category by itself. Lines were fit by a least squares linear regression.

On the basis of the niche overlap between the two silverside species and the results of our laboratory competition experiments, we hypothesize that *Menidia* competitively excluded *Labidesthes* from Lake Texoma. As observed in Lake Texoma, the *Labidesthes* population response to increased competition could be dramatic. *Labidesthes*, like *Menidia*, has an annual life cycle (Cahn 1927; Fogle 1959; Mense 1967; Hubbs et al. 1971), and its population is not buffered by multiple year-classes.

Despite the evidence supporting our hypothesis, we are

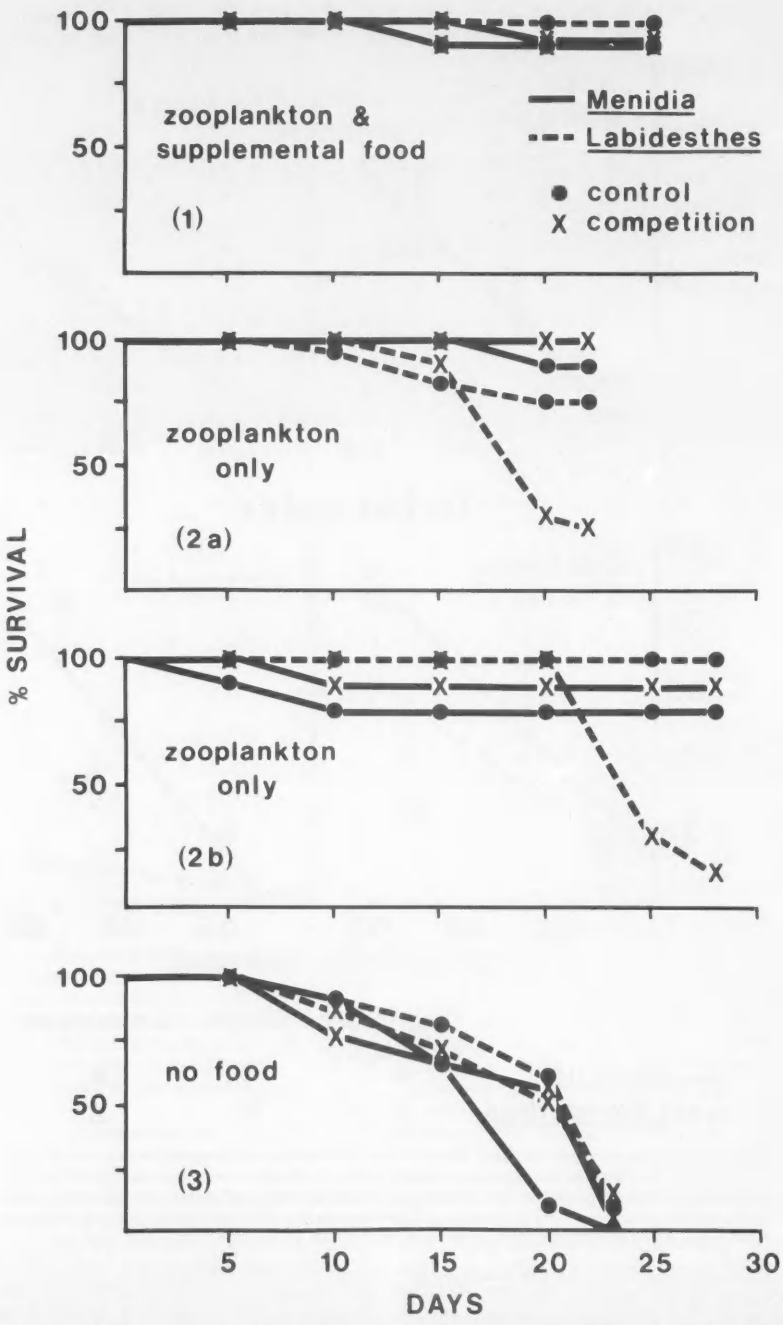

FIG. 4. Experiments testing the effects of food competition on young-of-the-year *Labidesthes* and *Menidia* survival.

unable to prove that competitive exclusion was responsible for the *Labidesthes* decline. We lack information about Lake Texoma during the replacement period and therefore cannot test our competition hypothesis or alternative hypotheses for the *Labidesthes* decline such as water quality changes or *Menidia* predation. In the future, ecologists may have an opportunity to test the competition hypothesis as *Menidia*, currently found in the lower part of the Mississippi River (Gilbert and Lee 1980), becomes introduced into lakes containing established *Labidesthes* populations. Whether *Menidia*'s introduction results in *Labidesthes*' exclusion or coexistence of the two species, such *Labidesthes*–*Menidia* interactions may provide a rare opportunity to evaluate the role of competition in the structuring of freshwater fish communities.

Acknowledgments

We acknowledge Dr Loren Hill for the use of the University of Oklahoma Biological Station facilities and the Environmental Protection Agency for use of the mobile research laboratory. We thank Drs Barry Chernoff, Anthony Echelle, Clark Hubbs, Michael McCracken, William Matthews, Don McNaught, and Stephen Threlkeld for helpful comments and reviewing drafts of the manuscript. This research was supported by a Texas Christian University Adkins Fellowship and by Texas Christian University Research Foundation Grant B7984.

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Improvements in Quantifying the Phosphorus Concentration in Lake Water

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Vertical and horizontal patterns in limnetic phosphorus concentrations ([P]) were detected in an oligotrophic lake by dividing the total phosphorus pool into two fractions: dissolved and particulate matter smaller than 250 μm (smaller fraction) and particulate matter larger than 250 μm (larger fraction). The smaller fraction was estimated from samples collected with a water bottle, and the larger fraction was estimated with tow net samples taken at several stations and to various depths. Our samples were digested with potassium persulfate which gave less variable results than other acid digestion techniques. The average variance associated with the mean [P] ($n = 3$) for samples collected and analyzed according to our procedure was less than 0.05 mg P/m^3. During summer stratification there was a consistent metalimnetic maximum in the smaller fraction, and there were small but significant differences in the concentrations found at two stations less than 1 km apart. During the same period the larger fraction was a significant portion (14–28%) of the phosphorus pool in the epilimnion which varied from 3 to 5 m in depth. It was a relatively constant portion of the phosphorus in the trophogenic zone (0–10 m) and in the 0- to 20-m portion of the water column i.e. 10–14% and 7.3–8.8%, respectively. In a separate experiment it was shown that by removing the larger fraction, the average variance associated with the mean [P] was reduced from 1.0, to 2.4×10^{-2} mg/m^3. This reduction occurred because the larger fraction contained zooplankters with relatively high but variable amounts of phosphorus, and which occur in densities too low to be adequately sampled with the smaller fraction.

Key words: phorphorus, lakes, phosphorus in zooplankton

PREPAS, E. E., AND F. H. RIGLER. 1982. Improvements in quantifying the phosphorus concentration in lake water. Can. J. Fish. Aquat. Sci. 39: 822–829.

Il a été possible de détecter la répartition verticale et horizontale des concentrations de phosphore limnétique ([P]) dans un lac oligotrophe en divisant la masse totale de phosphore en deux fractions : les matières dissoutes et particulaires de taille inférieure à 150 μm (petite fraction) et matières particulaires plus grosses que 250 μm (grosse fraction). La petite fraction a été estimée à partir d'échantillons recueillis à l'aide d'une bouteille à eau, alors que la grosse fraction a été estimée sur des échantillons prélevés au filet traînant à plusieurs stations et à diverses profondeurs. Nos échantillons ont été digérés au persulfate de potassium, ce qui donne des résultats moins variables que les autres méthodes de digestion acide. La variance moyenne associée au [P] moyen ($n = 3$) d'échantillons recueillis et analysés d'après notre méthode est inférieure à 0,05 mg P/m^3. À l'époque de stratification estivale, il y a maximum métalimnétique uniforme dans la petite fraction, et on observe des différences petites mais significatives entre les concentrations trouvées à deux stations à moins de 1 km de distance. Dans la même période, la grosse fraction représentait une portion significative (14–28%) de la masse de phosphore dans l'épilimnion, dont la profondeur variait de 3 à 5 m. Cette fraction constituait une portion relativement constante du phosphore dans la zone trophogénique (0–10 m) et dans la tranche de 0 à 20 m de la colonne d'eau, c.-à-d. 10–14% et 7,3–8,8% respectivement. Dans une autre expérience, il a été possible de démontrer qu'en enlevant la grosse fraction, la variance moyenne associée au [P] moyen diminuait, passant de 1,0 à

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Printed in Canada (J6552)
Imprimé au Canada (J6552)

2.4×10^{-2} mg/m^3. Ceci est dû au fait que la grosse fraction contient des zooplanctontes à teneur en phosphore relativement élevée mais variable, et qui sont présents à des densités trop faibles pour être adéquatement échantillonnés en même temps que la petite fraction.

Received June 25, 1981
Accepted February 15, 1982

Reçu le 25 juin 1981
Accepté le 15 février 1982

THE nutrient phosphorus is the basis of numerous widely used models to predict biomass and productivity in lakes (e.g. Dillon and Rigler 1974a; Hanson and Leggett 1981). In turn, a steady-state approach (Vollenweider 1969) is used to predict limnetic [P]. However, the values predicted by these models and observed phosphorus values often differ considerably (e.g. Reckhow 1977). The source of these differences may be poor estimates of phosphorus, or the models themselves. Unfortunately, much more time has been spent on developing the models which are used to estimate [P] (e.g. Snodgrass and O'Melia 1975; Scavia 1980) than improving the approach to collecting and analyzing water samples for phosphorus.

Marked vertical variation in lake water [P] has been reported (e.g. Zimmerman and Suter-Weider 1976), yet, there is no standard approach to sampling lake water to accommodate either vertical or horizontal gradients in [P]. Sampling depths vary considerably among authors, ranging from surface samples (e.g. Wright 1976) to integrated samples of the entire water column at the greatest depth (e.g. Ostrofsky 1978). Furthermore, data do not exist to determine the depths where samples should be taken, nor the number of locations required.

Similarly, the accuracy of traditional sampling techniques for estimation of phosphorus incorporated in various-sized particles found in lake water is unknown. In lake water samples large zooplankton and other similar-sized particles (referred to as the larger fraction) are either inadequately sampled or ignored. The quantity of lake water routinely used in chemical analysis, 50 mL, is often considered adequate to estimate the larger fraction (e.g. Ostrofsky 1978). However, as Chamberlain (1968) pointed out, a 50-mL sample is too small to estimate this fraction. Regardless of zooplankton concentration, subsamples from a water bottle will probably underestimate the larger fraction, due to avoidance of capture (Patalas 1951). The alternative approach to this fraction is to remove it from the water sample (e.g. Dillon and Rigler 1974a).

Concern about accurate estimates of the larger fraction would be unnecessary if the contribution of this fraction to the total phosphorus pool were negligible. However, the few existing studies have yielded conflicting and incomplete results. Hrbáček (1969) found that particles which were retained on a 50-μm net were 10% of total Kjeldahl nitrogen in fish ponds. Dillon and Rigler (1974a) found that particles larger than 216 μm constituted only a small portion (average 2%) of the total phosphorus pool in integrated samples taken throughout the water column at spring overturn, in 19 lakes in Haliburton, Ontario. However, in a subsequent study in the summer of 1976, Rigler (unpublished data) found that particles larger than 253 μm made up a significant but variable fraction (range 5–21%, average 14%) of the total phosphorus pool in the trophogenic zone of Bob Lake, one of the Haliburton lakes studied by Dillon and Rigler (1974a).

In this study we set out to determine whether a sampling program designed to obviate the effects of horizontal and vertical gradients in all phosphorus fractions, up to and including the largest fraction, could significantly improve whole-lake estimates of [P]. The study was carried out on Bob Lake where the [P] in lake water samples was as low as 5 mg/m^3. In order to be able to distinguish spatial gradients in lakes with such low [P]'s from sample variation, a modification of Menzel and Corwin's (1965) persulfate digestion technique was used.

Materials and Methods

Samples were collected from May until October 1978 at Bob Lake (44°54′N, 78°47′W), a relatively deep (mean depth 18 m), medium-sized (surface area 2.3 km^2), oligotrophic lake with a moderate flushing time (2.4 yr) in the Haliburton region of Ontario (Cross 1977). Water transparency was measured with a 20-cm diameter Secchi disk and the trophogenic zone was defined as two times Secchi disk depth. Total phosphorus was divided into two fractions: dissolved and particulate matter smaller than 250 μm (smaller fraction) and particulate matter larger than 250 μm (larger fraction). The mesh size (250 μm) used to separate the two fractions was chosen arbitrarily as the size of the smallest zooplankter which might be expected to escape a water bottle. Water samples for the smaller fraction were collected with a 1.5-L aluminum drop-sleeve water bottle. To test for horizontal and vertical differences we collected samples at two stations (north and center) ~ 0.8 km apart (max. depth 40–45 m), and at metre intervals from the surface to 15 m, and at 5-m intervals below 15 m. Stream water samples were collected directly in Nalgene bottles from the major Bob Lake inflowing and outflowing streams. Lake and stream water samples were placed in 1-L Nalgene bottles which were completely filled. Within 4 h these samples were filtered through a 250-μm Nitex net and transferred to culture tubes in which they were later digested.

The larger fraction, which consisted primarily of large zooplankton and phytoplankton, was sampled with a conical net 90 cm long with a 29-cm-diameter aperture. To avoid the possibility of the larger plankton migrating away from shadows caused by the boat, the boat was allowed to drift away from the station between samples. To estimate vertical distribution of this fraction, while minimizing the effects of horizontal gradients, vertical hauls were taken from several depths to the surface at three stations equally spaced along the north–south transect of the lake. Three stations may not have been adequate for estimating the larger fraction. However, data on horizontal distribution of this fraction are insufficient to develop an optimal sampling program. The samples were rinsed with double-distilled water (DDW) into 175-mL Nalgene bottles and frozen for up to 2 wk before analysis; hauls taken to the same depth from all three stations were pooled.

In each stratum, total phosphorus in the larger fraction was based on the difference between total phosphorus in the hauls that began at the bottom and top of that stratum. The contribution of the larger fraction to the total phosphorus pool was calculated as the ratio of [P] in the larger fraction to the sum of the smaller and larger fractions. To calculate the contribution of the larger fraction to several strata, the ratio for each stratum was volume-weighted. For example, on August 30 there were 0.9 and 0.7 mg P/m^3 in the 0- to 5 and 0- to 10-m hauls, respectively. Thus in the larger fraction there was 0.9×5 or 4.5 mg P/m^2 in the 0–5 stratum, 0.7×10 or 7 mg P/m^2 in the 10-m column, and by difference, 2.5 mg P/m^2 or 0.5 mg P/m^3 in the 5- to 10-m stratum. The measured [P] in the smaller fraction of the 0- to 5-m and 5- to 10-m strata were 4.8 and 6.5 mg/m^3, respectively. Thus $0.9 \div 5.7$ or 15.8%, and $0.5 \div 7.0$ or 7.1%, respectively, of the total phosphorus pool was tied up in the larger fractions. The relative volumes of the two strata were 1.0 and 0.79. Therefore the volume-weighted contribution of the larger fraction to the 0- to 10-m portion of the water column was 12.0%.

Total phosphorus was measured by a modified version of Menzel and Corwin's (1965) potassium persulfate technique which we will call the persulfate method. Glass culture tubes (25 mm × 200 mm) with polypropylene lids were used in place of borosilicate flasks, 0.4 g of potassium persulfate was added to each 50-mL sample, and digestion was carried out for 45 min. Lake and stream water and ground *Daphnia* samples from a laboratory culture were also digested by the perchloric acid method described by Strickland and Parsons (1972), modified by omitting the addition of potassium iodide and ammonia solutions and by performing the digestion in 125-mL Kjeldahl flasks. Because the ground *Daphnia* samples were rich in organic matter, 0.5 mL concentrated sulfuric acid was added to these samples in addition to the perchloric acid prior to digestion. Twenty-four hours after digestion they were neutralized with 1.5 mL 10 mol/L NaOH solution. The preparation of a mixed reagent was a modification of the method of Murphy and Riley (1962). A premixed solution (which remains stable for many months if protected from light) was prepared from 15 g ammonium molybdate, 0.34 g potassium antimony tartrate, 140 mL concentrated sulfuric acid, and 1650 mL DDW. When the samples were ready for analysis the mixed reagent was prepared from ascorbic acid, DDW, and premixed solution in the following proportions; 5.17 g, 100 mL, and 400 mL, respectively. To each sample we added 5 mL mixed reagent, then 30 min to 3 h later the absorbance at 885 nm was read in a Bausch and Lomb Spectronic 100 spectrophotometer with a 10-cm path length cell. All samples and standards were analyzed in triplicate. Thus each [P] reported is the mean of three samples. The glass culture tubes and Kjeldahl flasks were rinsed twice with DDW after use, and recapped. To remove phosphorus from new glass culture tubes and Kjeldahl flasks, they were filled with 50 mL DDW and subjected twice to the complete procedure for total phosphorus analysis. All other glassware and the Nalgene bottles used in the collection and analysis of total phosphorus were washed in 2% HCl acid, then rinsed four times with distilled water and three times with DDW.

TABLE 1. Mean phosphorus concentration (mg/m^3), ±SE in replicate samples of the larger fraction (>250 μm), ground and not ground before subsample taken for analysis.

Contents, m	Ground		Unground
	Prep 1	Prep 2	
0–4	7.0±0.05	6.9±0.05	7.6±1.13
0–6	10.6±0.10	10.7±0.05	9.1±0.60
0–10	11.7±0.13	11.9±0.08	12.7±0.87
0–20	16.4±0.10	15.6±0.05	15.2±0.76
0–20	14.5±0.10	14.7±0.10	14.4±0.56

Preparation of the Larger Fraction for Analysis

Samples to estimate the larger fraction were obtained by filtering from 0.32 to 10 m^3 of lake water. These samples contained as much as 7.2 mg P, or four orders of magnitude more P than a 50-mL sample of the smaller fraction. To keep the optical densities similar for digested samples from both fractions, the samples of the larger fraction were diluted, subsampled, and homogenized. The samples were made up from the initial volume to between 80 and 600 mL. A total of 3 to 8 mL was removed from the diluted sample, made up to 10 mL, and then ground to an emulsion in a Potter Elvehjem tissue grinder. Three subsamples of the emulsion were placed in culture tubes which were made up to 50 mL and analyzed for total phosphorus. These samples represented 0.025–0.625% of the original sample. All dilutions were made with DDW. The final [P] in these samples ranged from 6 to 36 mg/m^3. The initial dilution and subsample volume was determined from the volume of lake water filtered for the sample.

This method of sample preparation was very repeatable as illustrated in an experiment where duplicate sets of samples were prepared for analysis from samples taken to various depths. The results (Table 1) show no significant difference between replicate mean values (F-test, $P > 0.5$). The additional step of homogenizing the sample significantly reduced the sample variance, as illustrated when the same experiment was repeated but the samples were not ground (Table 1). The average variance was 1.96 mg P/m^3 for the unground samples and 2.16×10^{-2} mg P/m^3 for the ground samples (F-test, $P < 0.001$).

Calibration of Tow Nets

Samples to estimate the larger fraction were collected in May and early June with an 80-μm Nitex tow net attached to a modified Birge–Juday bucket made with 250-μm Nitex mesh, and the remaining samples were collected with a 250-μm Nitex tow net. To investigate whether the 80-μm and 250-μm tow nets both attached to a bucket made with 250-μm mesh collected comparative amounts of total phosphorus, we compared replicate tows on four separate dates. The overall difference in total phosphorus between the larger fractions collected by the two nets was less than 2%. Hence data collected with the two nets were comparable.

Prepas and Rigler (1978) showed that *Daphnia* can escape from an 80-μm Nitex tow net. Thus we compared the efficiency of the 250-μm tow net at collecting the larger fraction with a 32-L Schindler–Patalas Plexiglas trap fitted with 250-μm Nitex mesh. These comparisons were carried out on four separate days and at two locations in Bob Lake. Trap

TABLE 2. Ratio of phosphorus in the larger fraction (>250 μm) collected by the Schindler–Patalas trap to that collected by a tow net at various times at south (S) and central (C) stations in Bob Lake in 1978. Values adjusted for different volumes collected by the two sampling devices.

Depth (m)	Aug. 29, S 14:00–14:30	Aug. 31, C 10:00–14:30	Sept. 1, C 10:30–14:30	Sept. 1, S 16:00–18:00	Sept. 2, S 10:00–12:30
0–5	1.16	1.14	1.31	1.03	1.18
5–10		1.09	0.97	0.85	0.72
10–20		1.21	1.71		

samples were taken at 0.5-m intervals from 0.5 to 10 m and 1-m intervals from 11 to 20 m. The Schindler–Patalas trap is ~50 cm tall, thus all but the top 25 cm of the 0- to 10-m portion of the water column was sampled. Duplicate tow net samples were taken before and after each set of trap samples to allow for any migration of the animals up or down in the water column while the trap samples were being collected. Net efficiency was calculated by assuming the Schindler–Patalas trap was 100% efficient and comparing the amount of phosphorus collected by the trap with the amount of phosphorus collected by the tow net over the same depths. The different volumes of water filtered by the two devices was corrected.

The Schindler–Patalas trap collected more phosphorus per unit volume in 8 out of the 11 comparisons made (Table 2). The data were quite variable and we could not ascertain the cause. The trap did, however, sample less than a quarter of the volume of water sampled by the tow net in each set of comparisons and hence may have been more likely affected by plankton patchiness. On average, the Schindler–Patalas trap collected 12% more phosphorus than the tow net. Because there were no consistent trends with depth, all phosphorus values derived by the use of a tow net were increased by 12%.

Results and Discussion

An Evaluation of the Persulfate Method

The [P] in lake water samples from Bob Lake was often as low as 5 mg/m^3, which is lower than the 95% confidence intervals reported for the two standard digestion techniques; perchloric acid (Strickland and Parsons 1972), or a combination of sulfuric acid and potassium or ammonium persulfate (Jeffries et al. 1979; Environment Canada 1979; U.S.E.P.A. 1979). Thus to distinguish spatial gradients from analytical variation we required a technique which was more repeatable than the traditional techniques. We investigated whether the potassium persulfate technique described by Menzel and Corwin (1965) was suitable.

The potassium persulfate and perchloric acid digestion techniques were compared with water samples representing the range of samples collected from Bob Lake. The samples were analyzed in triplicate from depths of 2, 6, and 15 m, representing epilimnetic, metalimnetic, and hypolimnetic waters, respectively, and water from inflowing and outflowing streams. Persulfate digestion gave more repeatable results than perchloric acid digestion; the average variance in the [P] was 29 times higher for water samples and 20 times higher for standards digested with perchloric acid as compared with potassium persulfate (Table 3).

TABLE 3. Mean phosphorus concentration (mg/m^3) ±SE in water samples (smaller fraction) digested with either perchloric acid or potassium persulfate and analyzed in triplicate. Regression line statistics are for standards used to predict sample concentration.

Source	Perchloric acid	Potassium persulfate
Inflow	14.1±0.68	15.7±0.05
Outflow	5.4±0.13	5.8±0.00
Epilimnion	3.8±0.10	5.1±0.09
Metalimnion	5.5±0.23	6.5±0.09
Hypolimnion	4.6±0.23	5.6±0.05
Sample $\bar{S}^2$	3.534×10^{-1}	1.212×10^{-2}
Reg. line Intercept	4.07×10^{-3}	-2.0×10^{-4}
Slope	6.76×10^{-3}	6.65×10^{-3}
Reg. line Res. var.	5.24×10^{-4}	2.64×10^{-5}

The repeatability of potassium persulfate digestion is similar to that reported by Rigler (1979) for ultraviolet (uv) digestion. Rigler showed that standards digested with perchloric acid had a variance that was 20 times greater than if digested by uv, and the variance he reported for the mean optical density for standards digested by uv (1.44×10^{-6}) was remarkably similar to that which we found for standards of similar [P] digested with potassium persulfate (1.60×10^{-6}). However, Rigler noted that uv digestion consistently gives incomplete digestion and therefore was not used in this study.

The confidence intervals associated with mean [P] were an order of magnitude less variable with potassium persulfate digestion than those reported by Jeffries et al. (1979) where sulfuric acid was used in addition to potassium persulfate, and the samples were autoclaved. Jeffries et al. reported average 95% confidence limits of ±3 mg P/m^3 (excluding outliers) for lake water samples which ranged from 4 to 10 mg P/m^3, whereas we found that 95% confidence limits for samples digested with potassium persulfate averaged ±0.2–0.3 mg P/m^3 for samples which ranged from 4 to 50 mg P/m^3. Thus potassium persulfate digestion gave more repeatable results than any other digestion technique reported in the literature. The persulfate method gave results which would allow us to distinguish spatial gradients in lake [P].

In sediment samples (Menzel and Corwin 1965), and particulate matter originating from soils (O'Connor and Syers 1975), potassium persulfate reportedly gave incomplete phosphorus digestion. However, in all analyses of lake and stream

water samples in this study, potassium persulfate digestion extracted more (average 18%) phosphorus than perchloric acid digestion (Table 3). The lower recovery values for perchloric acid could be partially due to loss of phosphorus to the vessel walls during digestion. Chamberlain (1968) reported that 5% of phosphorus was lost in this way. To determine whether P was digested and analyzed equally well in lake water and DDW with the persulfate method, a standard addition experiment was performed. Duplicate sets of standards and blanks were prepared in volumetric flasks with DDW, and lake water collected from the trophogenic zone (0−10 m in depth) of Bob Lake. Triplicate samples from each flask were analyzed for total phosphorus following the persulfate method. The slope of the regression line of [P] on optical density for lake water, with 0, 10, 20, and 30 mg P/m^3 added, was not statistically different from the slope of the line for standards prepared with DDW (t-test, $P > 0.9$). The possibility of a hidden blank error was thus eliminated for the persulfate method. However, a hidden blank error cannot be discounted for perchloric acid digestion as no comparable data are available for this method. Although perchloric acid digestion extracted less phosphorus than potassium persulfate digestion, the sulfuric−perchloric method and the persulfate method extracted similar amounts of phosphorus from ground *Daphnia* (t-test, $P > 0.5$). Thus, when compared with perchloric acid digestion, the persulfate method completely digested the range of samples used in this study.

Spatial Variation in Phosphorus in the Smaller Fraction

A vertical phosphorus gradient in the smaller fraction was found on all occasions when the lake was stratified. As illustrated in Fig. 1, [P] in this fraction was lowest in the mixed layer, increased rapidly to a maximum which was almost double of that in the metalimnion, and decreased slightly in the hypolimnion. Other studies show that the [P] is higher in hypolimnia than epilimnia (e.g. Zimmerman and Suter-Weider 1976). However, we are not aware of any reports of a metalimnetic maximum. The metalimnetic phosphorus maximum observed at Bob Lake was always within the trophogenic zone and may coincide with a metalimnetic chlorophyll peak as described by Fee (1976). Thus, even in a relatively small oligotrophic lake there are vertical differences which should be considered in any whole-lake sampling program.

We also found horizontal differences throughout the stratified period between stations less than 1 km apart (Fig. 1). These differences were not entirely consistent; the epilimnetic [P] was lower at the northern station on three of the first four dates (two-way ANOVA, $P < 0.001$), equal at the two stations at the end of July ($P > 0.5$), and higher at the northern station in October ($P < 0.001$), whereas hypolimnetic [P]s were consistently higher at the northern station. The cause of these differences is unknown. The data suggest that more attention should be paid to horizontal variations in [P], even in relatively small oligotrophic lakes. To estimate [P] in the smaller fraction for Bob Lake, the data from the two stations were pooled.

The variance associated with mean [P] was substantially

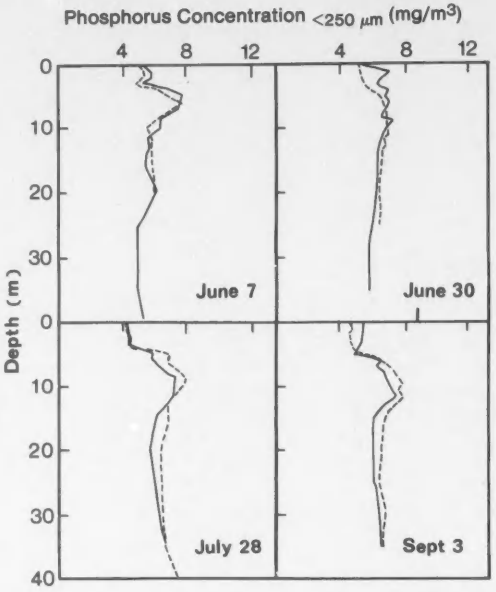

FIG. 1. Vertical distribution of phosphorus in the smaller fraction (<250 μm), at two stations on Bob Lake, 1978. Central (—), North (---).

reduced when the larger fraction was removed from water samples. To illustrate, 10 epilimnetic water samples were collected from Bob Lake, on two dates and at two stations. Half the water in each of the 10 samples was filtered with a 250-μm net, half was not filtered. The samples were then analyzed for mean [P]. As illustrated in Table 4, the average variance associated with the mean [P] decreased from 1.0 to 2.4×10^{-2} mg/m^3 (F-test $P > 0.001$), when the larger fraction was removed. The larger fraction adds variation because one large zooplankter contains considerable phosphorus rela-

TABLE 4. Mean phosphorus concentration (mg/m^3) ±SE of replicate samples of lake water. Half the water in each of 10 samples was filtered through a 250-μm net (smaller fraction) and half was not filtered (smaller + larger fraction). Samples were collected on two dates, at two stations, center (C) and north (N).

Date, station, and depth (m)			Filtered 250-μm net	Not filtered
Sept. 3	(C)	1	5.2±0.00	5.6±0.31
		2	5.1±0.09	5.6±0.58
Oct. 8	(C)	1	6.1±0.00	6.5±0.18
		2	6.2±0.18	6.9±0.68
		3	5.9±0.05	6.5±0.25
		4	5.8±0.05	8.0±1.30
Oct. 8	(N)	1	6.3±0.09	6.8±0.35
		2	6.2±0.10	7.2±0.67
		3	6.1±0.10	6.6±0.30
		4	6.0±0.09	6.5±0.05

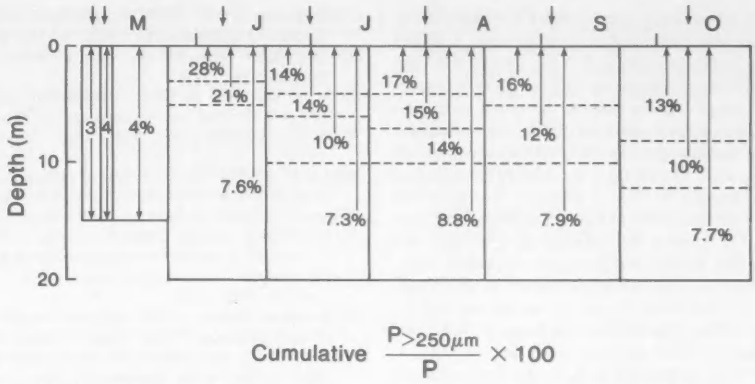

FIG. 2. Cumulative vertical distribution of the percentage of the total phosphorus pool (P) in the larger fraction ($P > 250$ μm) at Bob Lake, 1978, in an integrated sample from the surface to the depth indicated by the line under the percentage sign. Arrows indicate sampling dates.

tive to a 50-mL water sample from an oligotrophic lake. For example, Chamberlain (1968) reported that a single *Daphnia* contains as much as 4 mg P/m^3; whereas, the average [P] in the smaller fraction of Bob Lake was 6 mg/m^3. The inclusion of an invertebrate larger than a *Daphnia* could make the results impossible to interpret. For example, we found that one *Chaoborus* larva can add 100 mg P/m^3 to a 50-mL sample, or 17 times the average [P] found in the smaller fraction at Bob Lake.

Phosphorus in the Larger Fraction

The larger fraction was an important component of the total phosphorus pool of Bob Lake, particularly in the epilimnion where it was 13−28% of total phosphorus in the combined smaller and larger fractions. No metalimnetic maximum was observed, as in the smaller fraction, rather the contribution of the larger fraction decreased with depth. In the metalimnion it was 4−12% and in the hypolimnion it was from 3 to less than 1% of the total pool. The larger fraction was thus concentrated in the epilimnion while [P] in the smaller fraction was highest in the metalimnion. When the two fractions were added together the phosphorus gradient between epilimnetic and metalimnetic waters was much less than for the smaller fraction. This concentration of the larger fraction in the surface water explains why Dillon and Rigler (1974b), who estimated the larger fraction (>216 μm) from an integrated sample from the deepest part of the lake and did not volume-weight the sample, found it constituted less than 5% of the total phosphorus pool. To compare our estimated P in the larger fraction with Dillon and Rigler's, we assumed that they sampled to a depth of 40 m, and that the [P] in the larger fraction in the 20- to 40-m portion of the water column at Bob Lake was the same as in the 10- to 20-m portion, and we did not volume-weight the samples. The calculated contribution of the larger fraction to the total phosphorus pool in the 0- to 40-m portion of the water column from our data collected on Bob Lake was then 3−5% during summer stratification. These values are within the range reported by Dillon and Rigler. Dillon (1974) reported that relatively more phosphorus was incorporated in the larger fraction in shallow than in deep lakes. If the larger fraction is concentrated in the epilimnion of lakes, then Dillon's results could be due to the relatively greater volume of epilimnetic water in shallow lakes.

Other authors including Chamberlain (1968), Keup (1968), and Harris (1980) have suggested that the larger fraction constitutes a significant portion of the phosphorus pool in stream and lake water, yet this fraction is often ignored in nutrient budgets, because its contribution is considered variable (e.g. Dillon 1974). In contrast we found the contribution of the larger fraction was relatively constant over time. Figure 2 shows that from late June until early October, it was 13−17% of the epilimnetic [P] and 10−14% of the [P] in the trophogenic zone (0−10 m). In the 0- to 20-m portion of the water column, the larger fraction contributed a remarkably constant 7.3−8.8% of the total phosphorus pool, during the entire stratified period.

Results of a study done 2 yr earlier (Rigler unpublished data) on the same lake suggest that the contribution of the larger fraction may remain constant between years. In the earlier study Rigler found that an average of 14% of total phosphorus pool in the 0- to 10-m stratum was tied up in the larger fraction, which is very close to our average of 12% in the same portion of the water column. If the larger fraction remains relatively constant in a particular stratum within and between years, then it would be necessary to sample it only often enough to establish the vertical and seasonal patterns for the lake. However, more data are needed concerning within and between lake patterns for this fraction before any general statements can be made.

One possible explanation for the remarkable consistency in our estimates of the contribution of the larger fraction to the total phosphorus pool lies in our sampling approach. Because the larger fraction is often concentrated at one end of a lake (e.g. Langford and Jermolajev 1965), we sampled at several stations and thus improved our estimates severalfold. For example, in early May there was a strong southerly wind for

several days, and when we sampled on May 13, nearly all of the larger fraction was at the north end of the lake. Samples taken only at the center or south end of the lake on this date underestimated the larger fraction by 500 and 1200%, respectively, whereas samples taken only at the north end overestimated this fraction by more than 200%. To reduce the effects of horizontal heterogeneity, this fraction was sampled at three stations evenly spaced along the lake in the direction of the prevailing wind.

We found that the reliability of lake phosphorus estimates were improved by dividing the phosphorus pool into two fractions, the smaller fraction and the relatively mobile larger fraction. The latter was operationally defined as those particles which were collected by a tow net, and did not include organisms such as fish (Nakashima and Leggett 1980) and benthic invertebrates which migrate to the limnetic region. The inclusion of other organisms in the larger fraction could further improve whole-lake phosphorus estimates; however, data on their contribution must first be collected. More work should be done on lakes with different surface areas, mean depths, and trophic status to establish vertical and horizontal patterns for both fractions. These data could then be used to establish the number of sampling sites, and the depths to where samples should be taken for both fractions. Although additional information will undoubtedly improve our estimates of lake water [P], we found the sampling approach outlined here and Menzel and Corwin's (1965) digestion technique gave very repeatable results. For example, we calculated confidence intervals for the epilimnetic [P] in the smaller fraction which combined between and within-station variation. The 95% confidence intervals for any 1 d ranged from $\pm 0.3-0.1$ mg P/m^3 ($n = 30-36$).

More precise phosphorus estimates can be used to improve existing models to predict [P] in lake water and develop new ones. For example, current models predict annual average [P] from annual average inputs to, and outputs from, the lake. Recently, Prepas and Rigler (1981) suggested that better predictions of the [P] in lake water could be made if seasonal variations in the inputs to, and outputs from, the lake were incorporated into whole-lake models. The seasonal approach has also been used to estimate return of phosphorus from sediments of enriched lakes (Vollenweider 1976; Larsen et al. 1979). There are few data sets accurate enough to test a seasonal model, either to predict seasonal changes in limnetic [P] or to estimate a parameter such as return of phosphorus from the sediments of enriched lakes by the difference between inputs to and outputs from the lake. However, we have illustrated a method for collection and analysis of samples which produces data accurate enough to test a seasonal model, even on an oligotrophic lake such as Bob Lake where seasonal changes are relatively small.

Acknowledgments

We thank A. M. Zimmerman, L. Butler, and D. Liversage for their encouragement and support during this study; J. R. Nursall, C. J. Strobeck, H. Hamilton, and D. Trew for reviewing the manuscript; W. M. Chamberlain for his comments; and the National Research Council of Canada for financial support in the form of a postgraduate scholarship to the principal author, and operating grants to F. H. Rigler.

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Hydrocarbons Depress Growth and Reproduction of *Daphnia pulex* (Cladocera)

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GEIGER, J. G., AND A. L. BUIKEMA JR. 1982. Hydrocarbons depress growth and reproduction of *Daphnia pulex* (Cladocera). Can. J. Fish. Aquat. Sci. 39: 830 – 836.

Daphnia pulex were acutely and chronically exposed to water-soluble fractions of hydrocarbons. In acute studies, the most-to-least toxic were coal-tar creosote, No. 2 fuel oil, naphthalene, and phenanthrene. During chronic studies, *Daphnia* were monitored for molting frequency, growth rates, production of total and live young, number of nonviable eggs, partial and full abortions, and whether or not abortions occurred prior to completion of embryonic development. For creosote and phenanthrene, marked reduction occurred in growth rates, number of broods, and impairment of molting, and an increase occurred in abortion rates. No. 2 fuel oil produced similar effects, but results were less significant. Naphthalene only produced a slight reduction in growth rate at the concentrations studied. It is hypothesized that the effect on growth and reproduction may be due to PAH-induced disturbance on some aspect of metabolic control of reproduction and molting.

Key words: phenanthrene, naphthalene, creosote, No. 2 fuel oil, *Daphnia*, toxicity

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Des daphnies (*Daphnia pulex*) ont été soumises à des expositions aiguës et chroniques de fractions d'hydrocarbures solubles dans l'eau. Dans les expositions aiguës, les hydrocarbures par ordre croissant de toxicité sont la créosote du goudron de charbon, le mazout n° 2, le naphthalène et le phénanthrène. Dans les expositions chroniques, les daphnies ont été suivies en vue d'observer la fréquence des mues, le taux de croissance, le production du total des jeunes et des jeunes vivants, le nombre d'oeufs non viables, les avortements partiels et complets, et aussi en vue de découvrir si oui ou non les avortements ont lieu avant la fin du développement embryonnaire. Dans le cas de la créosote et du phénanthrène, il y a une diminution marquée du taux de croissance, du nombre de portées et des irrégularités de la mue. Il y a par contre augmentation des taux d'avortement. Les effets du mazout n° 2 sont identiques mais moins significatifs le naphthalène, aux concentrations utilisées, ne cause qu'une légère diminution du taux de croissance. Nous émettons l'hypothèse que l'effet sur la croissance et le reproduction serait dû à un dérangement provoqué par le PAH sur un aspect quelconque du contrôle métabolique de la reproduction et de la mue.

Received August 28, 1981
Accepted March 9, 1982

Reçu le 28 août 1981
Accepté le 9 mars 1982

AVAILABLE literature on acute toxicity of hydrocarbons to marine and estuarine organisms has been reviewed extensively (Moore and Dwyer 1974; Weiss 1976; Lockwood 1976; Reish et al. 1979). However, few researchers have investigated biological effects of these compounds in freshwater. Shelford (1917), one of the earliest workers, detailed various physiological and behavioral changes in several species of freshwater fish exposed to gas waste. Several investigators examined the use of *Daphnia* in toxicity testing of oil and oil emulsifiers (Dowden 1965; Grodner 1959; Rothschein 1970), refinery wastes (Dorris et al. 1974), and petroleum-related industrial wastes (Anderson 1944; Anderson et al. 1948). More recently, Buikema et al. (1976) found *Daphnia pulex* the most sensitive organism tested using a simulated refinery effluent. Burks and Wilhm (1977) tested a multispecies response using benthic invertebrates to evaluate various refinery wastewaters.

The importance of algae as primary producers has led to several reports on both acute and chronic toxicity of petroleum components on algal growth, uptake, and photosynthesis (Soto et al. 1975a, b; Kauss and Hutchinson 1975; Bott and Rogenmuser 1978). We investigated the biological effects of petroleum products and coal-tar creosote on the next link in the food chain using a common freshwater zooplankter.

Materials and Methods

GENERAL

The test organism was *D. pulex*, a common freshwater cladoceran, obtained from cultures maintained for several years in our laboratory. Both stock and experimental animals

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Printed in Canada (J6604)
Imprimé au Canada (J6604)

were kept in Sherer CEL 4-4 environmental chambers at 20 ± 1.5°C on a 16L:8D photoperiod with ~ 1100 lx at the air–water interface of the test containers. Light was provided by G. E. cool-white deluxe fluorescent bulbs. All stock cultures were fed *Chlamydomonas reinhardi* (wild type, negative strain) ad libitum, and approximately once a week their diet was supplemented with a trout chow–cerophyll extract. Algae were cultured in a modified Bolds basal medium (Buikema 1970), centrifuged, and resuspended in tap water before being fed to *Daphnia*. Prior to use as dilution water, Blacksburg tap water was carbon-dechlorinated and millipore-filtered (0.45 μm). During the lifetime exposure study, *Daphnia* were fed algae at a concentration of 45 000 cells/mL; algal concentrations were determined with an Electrozone particle counter.

Test Chemicals

American Chemical Society grade naphthalene (0.034 g/L) and phenanthrene (1.0 g/L) crystals were washed, then placed in filtered and dechlorinated tap water prepared as above for algae, and heated to 80 and 100°C, respectively. The suspensions were swirled gently until crystals were dissolved, and then the solutions were slowly cooled to room temperature. Solutions were then millipore-filtered to remove undissolved crystals, and the stock water-soluble fractions (WSF) were utilized to obtain test concentrations. In addition, millipore-filtering and heating helped eliminate potential degradation and/or transformation of the test compounds by fungi and bacteria (Cerniglia et al. 1978). No. 2 fuel oil (Gulf Oil Company, Philadelphia, PA) and coal-tar creosote (Ace Hardware) WSFs generally were prepared according to the method of Anderson et al. (1974). For these extractions, one part oil or creosote was combined with nine parts of water and stirred for 20 h at room temperature (20 ± 2°C). After mixing, the oil and water were allowed to separate before the water was siphoned off. Extraction water was filtered, dechlorinated tap water. The WSF phase was millipore-filtered before use in toxicity tests. For acute tests, new solutions were made for each test. For chronic tests, new solutions were made every 3 d and stock WSFs were stored at 4°C.

Early in the study, WSFs were examined by gas chromatography. Portions of 100 mL of each freshly prepared WSF were extracted three times with a total of 100 mL pesticide-grade pentane. Extracts were combined, dried over anhydrous Na_2SO_4, and evaporated nearly to dryness under a stream of nitrogen. Hydrocarbons were analyzed on a Varians 2440-MAT112 gas chromatograph–mass spectrophotometer with FID detectors. A 1.5 m × 2 mm (ID) glass column packed with 3% SP-2100 on supelcon AW-DMCS was used. Carrier gas was helium (30 mL/min), and detector gasses were air (300 mL/min) and hydrogen (30 mL/min). Oven temperatures were programmed from 90 to 275°C at 4°C/min. Peak areas were quantitated to injected standards of phenanthrene and naphthalene. The amount of phenanthrene and naphthalene present in the WSF was 0.96–1.28 mg/L and 5.08–6.76 mg/L, respectively. Analysis of No. 2 fuel oil and creosote WSFs indicated the presence of up to 40 individual peaks in each WSF (Geiger 1979).

Acute Tests

Acute tests were usually conducted using 500-mL pyrex beakers with five neonate *Daphnia* per 300-millilitre test solution. Tests were static without renewal. Air was supplied with disposable Pasteur pipettes so that bubbles were produced ~ 2–4 times per second (Anderson et al. 1974) to prevent any possible oxygen depletion. Animals were not fed during testing. Water hardness, alkalinity, and pH of dilution water averaged 43 and 45 mg/L and 7.2, respectively. Organisms were examined at 8, 24, and 48 h, and mortality was recorded. At least 25 animals were exposed to each concentration, including the control. On rare occasions, control mortality exceeded 10%, and these test results were discarded. Data were pooled, and LC50 values with appropriate confidence intervals were computed using probit analysis (Barr et al. 1976).

Chronic Tests

From these acute dose/response curves, the percent concentration of WSFs corresponding to the LC20 and LC30 were determined and utilized for chronic tests. Tested concentrations are denoted throughout the text as the abbreviation of the test WSF compound with a 2 or 3 to designate the respective LC20 or LC30 concentration (i.e. creo-2 refers to creosote LC20 concentration). These tested values (in % WSF) were as follows: nap-2 (5.6), nap-3 (10), phe-2 (10), phe-3 (32), oil-2 (5.6), oil-3 (10), creo-2 (1.0), and creo-3 (1.8).

Each chronic experimental group started with 10 neonates 24 ± 12 h old per test concentration. The control group consisted of 20 organisms. Test solutions were renewed daily.

Dissolved oxygen, alkalinity, hardness, and pH were determined weekly using standard procedures (American Public Health Association et al. 1975). Throughout all tests, dissolved oxygen varied from 7.5 to 9.0 mg/L; alkalinity from 40 to 47 mg/L (as $CaCO_3$); hardness (as $CaCO_3$) from 41 to 50 mg/L; and pH from 6.9 to 7.5.

Animals were maintained individually in glass baby food jars with 50 mL test solution. Animals were fed daily 45 000 cells/mL and examined for molting, live young, number of dead young (with and without caudal spines), number of nonviable eggs, and partial and full abortions. If they had molted, body length was measured (as determined by ocular micrometer from top of head to base of caudal spine). The presence of dead young without caudal spines indicated premature ejection of an incompletely developed organism; dead young with spines implied that the organism was completely developed and would normally have been released by the

TABLE 1. Acute LC50 values for *Daphnia* exposed to the water-soluble fractions of organic compounds.

Compound	48-h LC50	
	% WSF (± C.I.)	mg/L[a]
Naphthalene	57.52 (46.02–76.79)	2.92–3.89
Phenanthrene	⩾100	0.96–1.28
No. 2 fuel oil	34.10 (27.94–41.78)	—
Creosote	2.91 (2.52–3.36)	—

[a]Determined by gas chromatography of stock solution.

TABLE 2. Reproduction data resulting from chronic exposure of *Daphnia pulex* to four hydrocarbon WSFs. Data are mean values (±SD).

Compound	(*n*)	Estimated concn.	Live young	Dead young (w/spine)	Dead young (w/o spine)	Broods/animal	Live young/brood/animal	Total dead/animal	Partial abortions	Full abortions	Total abortions	Survival (d)
Control	(19)	—	252±155	0.7±1.4	25.9±12.7	16.4	15.4	26.6	0.6±0.7	0.8±1.1	26	57.4±30.6
Oil-2	(7)	20% WSF	252±116	1.3±2.2	53.1±24.2	20.0	12.6	54.4	1.4±1.0	1.9±1.1	23	69.3±26.8
Oil-3	(9)	30% WSF	199±113	0.4±1.3	39.7±23.0	16.3	12.2	40.1	1.7±1.4	1.0±1.2	24	56.1±18.1
Creo-2	(10)	20% WSF	133± 77	1.8±3.7	93.0±40.4	20.8	6.4	94.8	3.8±3.0	4.6±1.6	84	72.5±19.8
Creo-3	(10)	30% WSF	7± 12	0.2±0.4	38.2±30.0	9.6	0.7	38.2	6.2±5.2	0.7±1.6	69	42.8±25.1
Phe-2	(10)	0.11 mg/L	221±134	0.4±1.0	12.9± 8.9	19.1	11.6	13.3	0.5±0.5	0.6±0.7	11	67.3±35.3
Phe-3	(9)	0.36 mg/L	45± 28	0.1±0.3	2.2± 2.6	12.0	3.7	2.3	0.5±0.7	0.0±0.0	5	51.6±29.3
Nap-2	(9)	0.33 mg/L	314± 91	3.6±4.6	28.0±24.0	25.8	12.2	31.6	0.7±1.0	1.3±2.1	18	90.1±23.4
Nap-3	(8)	0.60 mg/L	244±140	0.2±0.7	9.4±10.4	23.4	10.4	1.6	0.5±1.1	0.5±0.8	8	84.0±41.3

female within a short period of time. A partial abortion was considered premature ejection of a portion of a newly developing brood from the brood chamber; a full abortion was the premature ejection of the entire developing brood.

DATA ANALYSIS

Data were analyzed by brood using SAS76 General Linear Model Analysis of Variance and Duncan's Multiple-Range Test procedures (Barr et al. 1976). Animals accidentally killed during these experiments were not included in statistical analyses. Statements of significance refer to $P < 0.05$.

Results and Discussion

ACUTE TEST

Creosote was the most toxic of the four WSFs tested (Table 1). In creosote, most deaths usually occurred within the first 8 to 24 h. No. 2 fuel oil also was quite toxic, and mortality was most pronounced within the first 24 h. Naphthalene toxicity was low compared to creosote and No. 2 fuel oil. A 100% phenanthrene WSF was not acutely toxic in 48 h.

Animals were not removed from test containers for the duration of each test. Several times, especially for naphthalene and creosote, animals would appear to be dead, but they later recovered. This "narcotic" effect has been noted also for marine invertebrates (e.g. Crisp et al. 1967; Berdugo et al. 1977; Sanborn and Malins 1977) exposed to various organic compounds.

Phenanthrene was not expected to demonstrate much of an acute effect. Although an LC50 could not be calculated by probit analysis, sufficient data existed to permit calculations of approximate LC20 and LC30 concentrations. Analysis of phenanthrene stock WSFs by GC-MS confirmed that phenanthrene was in solution.

CHRONIC TESTS

At both concentrations of phenanthrene, the effects were different from controls and usually from each other (Tables 2 and 3). The phe-2 population lived longer, produced more broods per animal, and less total live young than did the controls, but the differences were not significant (Table 2). The phe-2 organisms produced more dead young without spines than the phe-3 group. The phe-3 population died sooner than the phe-2 or control groups. The phe-3 population produced significantly fewer broods per animal and fewer live young per brood than either the phe-2 or control animals. Phe-3 organisms also reached reproductive maturation ~3 d later than did either controls or the phe-2 animals (Table 3). Similar delays in development rates of organisms exposed to petroleum hydrocarbons have been reported for decapod crustaceans (Katz 1973; Wells and Sprague 1976; Caldwell et al. 1977; Laughlin et al. 1978), marine amphipods (Linden 1976), mud crabs (Laughlin and Neff 1979), and Pacific herring larvae (Struhsaker et al. 1974).

The creo-3 organisms had a shorter survival time and more partial abortions, and significantly fewer broods and live young per animal than did the controls (Table 2). Creo-3

TABLE 3. Onset of reproductive maturation of *Daphnia pulex* exposed to water-soluble hydrocarbons.

Test compound	Size at first brood (mm) (mean±SD)	Appearance of the first brood (d)[a] (mean±SD)
Control	1.79(±0.13)	7.2(±0.5)
Oil-2	1.76(±0.12)	6.7(±0.5)
Oil-3	1.63(±0.16)	7.3(±0.5)
Creo-2	1.55(±0.13)	7.1(±0.3)
Creo-3	1.43(±0.11)	9.2(±1.1)[b]
Phe-2	1.60(±0.08)	7.0(±0.7)
Phe-3	1.31(±0.06)	10.8(±1.0)[b]
Nap-2	1.75(±0.12)	7.6(±0.5)
Nap-3	1.66(±0.11)	7.7(±0.8)

[a]Organisms were 24 ± 12 h old at the beginning of the chronic studies.

[b]Significantly different ($P < 0.05$) by Duncan's procedure.

TABLE 4. Number of times experimental populations were significantly different from control populations for the variables body length and number-of-the-young based on a brood-by-brood comparison.

Compound	No. of broods compared[a]	Length of adults	No. of broods compared	No. live young
Oil-2	25	0	25	12
Oil-3	25	24	25	9
Creo-2	25	25	25	19
Creo-3	21	21	20	19
Phe-2	25	25	25	12
Phe-3	20	20	20	19
Nap-2	25	24	25	13
Nap-3	25	25	25	14

[a]Where insufficient data existed, broods, especially those near the end of the life cycle, were not compared when n became small.

significantly delayed the onset of reproductive maturity (Table 3). Creo-2 populations lived longer, and produced a significantly higher number of broods, live young per brood, and full abortions than the creo-3 organisms. Additionally, creo-2 organisms exhibited the highest number of dead young without spines than any other WSF tested.

Both populations exposed to naphthalene lived significantly longer and produced an equal or greater number of live young than did the controls. This phenomenon has been noticed by other workers (Smyth 1967; Eldridge et al. 1977). Smyth (1967) speculated that exposure to small chronic levels of a toxicant may stimulate adaptive physiological functions which help a healthy organism maintain homeostasis. For all experimental animals, the longest living organism was one exposed to an LC30 concentration of naphthalene. This female lived for 125 d and produced young up to day 121. Filtration rate and oxygen consumption experiments (Geiger and Buikema 1981) indicate that organisms exposed to naphthalene exhibited a reduction in metabolism; the energy conserved may account for lengthened life span.

Oil-2 organisms had the second highest number of mean dead without spines and total dead per animal of all compounds tested. However, *Daphnia* exposed to oil-3 did not exhibit significant differences from control organisms. This dichotomy between LC20 and LC30 effects on dead young without spines was observed for every WSF studied (Table 2). Except for creosote, the LC20 concentration had the highest number or proportion of dead young without spines. These results may reflect a possible consequence of detoxification. Oxidation of these hydrocarbons by mixed function oxidase systems may enhance cytotoxicity; such oxygenated derivatives have been implicated in producing genetic damage in mammals (Varanasi and Malins 1977).

The most significant effects caused by all WSFs were on body length and number of live young when data were compared by brood for the first 20 to 25 broods of surviving animals (Table 4). Except for oil-2 populations, body length was significantly reduced by the materials studied. For oil-3 and nap-2, the effect on length did not appear with the second brood. Although brood by brood comparison of mean size indicated that controls were always larger than oil-2 organisms, it was not significantly different. Based on number of live young per brood, significant reductions in reproduction were observed 36 to 95% of the time. Most comparisons between the control and creo-2 and creo-3 and phe-3 were significantly different. These effects were similar to those observed for impairment of total live young production (Table 2). Most important, these data indicate that body length may be a more sensitive indicator of sublethal effects than is production of live young. Similar results have been reported by Geiger et al. (1980) and Winner (1981).

Most animals that were unable to molt successfully died during the chronic study. This was particularly true for phe-3 and creo-3. Also, one organism from each of these two groups developed an unusual dark wart-like growth under the exoskeleton of the organism. This "growth" (probably due to chitinoclastic bacteria) eventually inhibited molting.

Water-soluble fractions of oil are richer in aromatic hydrocarbons than are the original parent oils (Blumer et al. 1970; Boylan and Tripp 1971; Anderson et al. 1974). Pancirov and Brown (1975) found several polynuclear aromatic hydrocarbons (PAHs) in No. 2 fuel oil, and phenanthrene (and methylphenanthrene) was prominent. Similarly, Nestler (1974) describes whole coal-tar creosote as being ~90% PAHs. Aromatic hydrocarbons and PAHs are generally responsible for most of the toxicity of petroleum and its refined products. GC−MS spectra confirmed the presence of several PAHs in creosote. This could be the major reason for the substantial chronic effects of creosote on growth and reproduction of *D. pulex*.

The chronic effects of petroleum hydrocarbons on growth and reproduction of marine organisms have been documented by several workers. The hatching rate of grass shrimp larvae was reduced by petroleum hydrocarbons, and postexposed gravid females held in clean seawater had significantly lower number of larvae (Tatem 1977). Life span, total egg production, mean brood size, and rate of egg production were significantly reduced when marine copepods were exposed to WSFs of heating oil (Berdugo et al. 1977). Duration of four zoeal stages of mud crabs was significantly increased by exposure to increasing concentrations of WSFs of No. 2 fuel oil (Laughlin et al. 1978). Dungeness crab zoea chronically exposed to naphthalene had slightly increased duration of the first three zoeal stages (Caldwell et al. 1977). Phenanthrene decreased and naphthalene increased the development rates of

mud crab larvae (Laughlin and Neff 1979). Juvenile shrimp exposed to WSFs of crude oil at three different temperatures exhibited a reduction in growth rate and respiration (Edwards 1978).

Several important aspects of these data must be considered in attempting to explain the growth and reproduction effects of these WSFs on *D. pulex*. The creo-3 and the phe-3 concentrations resulted in the most dramatic reduction of growth and reproduction; they also significantly delayed the onset of reproductive maturation. Organisms that died during the chronic study because of their inability to molt were usually found in creo-3 and phe-3.

The above results indicate a disturbance of both the reproductive and molting processes. Molting and reproduction dominate the life cycle of *Daphnia*, and these events are intimately related (Lee and Buikema 1979). In crustaceans, both processes are mediated by steroid hormones or precursors (Highnam and Hill 1969; Novales et al. 1973; Teshima 1972) most probably obtained from the diet (Alam et al. 1979).

Recently O'Hara et al. (1978, 1979) documented the presence of a steroid in the marine copepod *Calanus* that binds with mammalian estradiol 17-β antibody. This datum indicates the presence of either 17-β or a very similar steroid in *Calanus*, and it may be involved in reproduction. Recently, Takeda (1979) found that certain estrogens (including estradiol 17-β) stimulated egg laying in several species of slugs. Current evidence indicates that in mammals the initial step of estrogen action involves binding of estradiol 17-β to cytosolic receptors that in turn is translocated into the nucleus where the estrogenic message is expressed (Kupfer and Bulger 1976).

O'Hara et al. (1978) point out that metabolism of PAHs and steroid metabolism may utilize the same mixed function oxidase systems. If one assumes the presence of a functioning endocrine system in *D. pulex*, the dietary intake of steroids from algae, and subsequent production of analogous molting hormone (crustecdysone) and estrogen-like hormone (estradiol 17-β), then a mechanism for the observed responses of *D. pulex* to some of the WSFs may be hypothesized. Target cells for any hormone contain specific hormone receptors located either on the cell surface or in the cytosol, depending upon whether the hormone is water-soluble or lipid-soluble; binding of the hormone receptor causes the formation of an intracellular messenger molecule (i.e. cyclic AMP, or others) that then stimulates or depresses some biochemical activity in the target cell (Lehninger 1975). Phenanthrene and a typical steroid have very similar molecular structures, and steroids are indeed modified derivatives of perhydrocyclopentanophenanthrene (Lehninger 1975). Phenanthrene (or similar polynuclear aromatic hydrocarbons) may either interfere with normal steroid metabolism by causing alterations in, or inhibit binding to, specific receptor proteins, or "mimic" endogenous steroid activity, influencing protein and glucose metabolism (reducing glucose availability for energy production as well as proteins available for growth) and lipid synthesis and/or mobilization (causing reduced egg production and decreased viability). These PAHs could also induce mixed function oxidase activity, resulting in less endogenous hormones which could result in metabolic malfunctions being translated into severe impairment of growth and reproduction.

Recent evidence supports this hypothesis. Estradiol 17-β administered in vivo at physiological concentrations to *Mytilus edulis* has been implicated in inducing a reduction in lysosomal stability in digestive cells; this indicates a hormonally controlled mechanism for cellular catabolism (Moore et al. 1978). PAH interference with hormone-mediated catabolism would have dramatic implications for growth as well as for reproduction. Energy normally produced from food sources would not be available for vital metabolic processes due to either incomplete digestion (catabolic malfunction) or components not being assimilated in usable form. Secondary effects from PAH interference with mixed function oxidase (MFO) activity could result in a toxic reaction. Introduction of molecular oxygen by MFOs could increase cytotoxicity or cause genetic damage and be responsible for the embryological effects shown by this study.

Another factor may also cause increased reproductive success of *Daphnia* exposed to low concentrations of WSFs. Hydroperoxides were formed after irradiation of No. 2 fuel oil with simulated sunlight (Larson et al. 1977). Addition of hydrogen peroxide to seawater caused synchronous spawning in gravid male and female abalones, as well as in other mollusks (Morse et al. 1977). Morse suggested that peroxide activated the enzyme prostaglandin endoperoxide synthase (PES) and that the synthesis of PES within eggs may play an essential role in early embryonic development. Prostaglandins, cyclic derivatives of unsaturated fatty acids, are found in many animal tissues and exhibit a great variety of hormone-like physiological and pharmacological effects. Morse et al. (1978) found peroxide-activated prostaglandin synthase in the reproductive tissues of a wide variety of marine organisms, including crabs and fishes. *Daphnia* may also possess a similar PES system. Phototransformation of hydroperoxides resulting from uv irradiation of refined oil fractions could induce PES and increase reproductive success (Morse et al. 1977).

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Comparison of Two Methods of Combining Catch-Per-Unit-Effort Data from Geographic Regions

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We examined two methods of combining catch-and-effort data from small geographic regions into large regulatory areas to estimate catch-per-unit-effort (CPUE): (1) weighting regional CPUE by bottom area occupied by the population; (2) weighting by fishing effort. Variance estimates and other statistical properties are presented for CPUE estimates by region and for the two methods, for the type of fishery where total catch is known. The CPUE estimator from area-weighting is approximately unbiased as an index of population density; the CPUE estimator from effort-weighting is biased but less variable than the former. Conditions of similarity are derived for estimators from the two methods to be identical, or, less restrictively, to show the same trend over time. In general, the area-weighted method is preferred, if sufficient samples are taken from all pertinent regions in the regulatory area. However, the effort-weighted method may be used with a gain in precision and no substantial bias, if there are no substantial changes in relative CPUE and effort among regions, or if relative CPUE and effort are inversely related. The two methods applied to catch and effort data for Pacific halibut (*Hippoglossus stenolepis*) and to produce highly similar CPUE estimates over time, despite large relative changes in both CPUE and effort among regions. The reasons for the similarity are traced back to conditions in the fishery.

Key words: catch-per-unit-effort, combining data, sampling commercial fisheries, variance estimation, comparison of methods

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Pour estimer les prises par unité d'effort (PUE), nous avons étudié deux façons de combiner les données sur les prises et l'effort de régions géographiques limitées pour former des zones sujettes à la réglementation plus étendues: (1) pondération des PUE régionales par superficie de fond occupée par la population; (2) pondération par effort de pêche. Nous présentons des estimations de variance et autres propriétés statistiques des estimations de PUE par région et par ces deux méthodes, et pour le type de pêche dont les prises totales sont connues. L'estimateur des PUE après pondération par âge est à peu près dépourvu de biais comme indice de densité de population; celui obtenu par pondération par l'effort est biaisé mais moins variable que l'autre. On constate que les conditions de similitude des estimateurs avec les deux méthodes sont identiques ou, de façon moins restrictive, montrent la même tendance avec le temps. En général, on préfère la méthode de pondération par région, si on dispose d'un nombre suffisant d'échantillons de toutes les régions pertinentes dans la zone sujette à la réglementation. Cependant, on y gagne en précision, sans biais substantiel, avec la méthode de pondération par effort s'il n'y a pas de grands changements des PUE et de l'effort relatifs au sein des régions, ou si les PUE et l'effort relatifs sont en corrélation inverse. Nous avons appliqué les deux méthodes à des données de prises et d'effort de pêche du flétan du Pacifique (*Hippoglossus stenolepis*). En dépit d'importants changements relatifs à la fois des PUE et de l'effort parmi les régions, nous avons obtenu des estimations de PUE assez identiques sur une période de temps. On a constaté que les conditions de la pêche étaient responsables de cette ressemblance.

Received August 11, 1981
Accepted February 15, 1982

Reçu le 11 août 1981
Accepté le 15 février 1982

Printed in Canada (J6584)
Imprimé au Canada (J6584)

CATCH-per-unit-effort (CPUE) is a reliable index of population density only if assumptions regarding fishing gear and spatial and temporal distributions of fish and fishing effort are satisfied (Beverton and Holt 1957; Gulland 1969, p. 44–57; Ricker 1975, p. 18–27). When fishing gear and temporal effects have been fully standardized, the resulting CPUE should be proportional to the average population density. A population, however, is usually distributed with variable densities over several fishing grounds.

Rarely can sufficient information about catch and effort be collected on all fishing grounds. Regions are defined as geographic areas encompassing several fishing grounds in close proximity. Sample catch and effort data for a region are extrapolated to determine total catch and effort.

We examined two methods of combining catch-and-effort data over regions into larger regulatory areas to estimate an annual index of average population density. The first method, called area-weighting, interprets regional CPUE as an index of population density and weights regional CPUE by the relative bottom area of each region (Helland-Hansen 1909; Widrig 1954; Paloheimo and Dickie 1964; Gulland 1969; Ricker 1975). The second method, called effort-weighting, divides the combined catch by the combined effort across regions which equivalently weights each regional CPUE by its total fishing effort (Gulland 1969).

The statistical properties of the CPUE estimates from the two methods are examined, and hitherto unpublished estimators of variance are developed. Statistical properties of the methods are compared, and "conditions of similarity" are derived which produce: (1) identical estimates, or (2) estimates that are different but show the same trend over time. Finally, the methods are applied to CPUE data from two regulatory areas of the Pacific halibut (*Hippoglossus stenolepis*) fishery. The results are discussed in relation to the theoretical principles established, and recommendations are given for the general use of the two methods.

Regional Estimates of CPUE

Although procedures for obtaining catch-and-effort data are not well documented, a survey of the literature of major fisheries suggests that usually catch or landings (C) are known without significant error and either CPUE or effort (E) is estimated (Gulland 1969, p. 44; Ricker 1975, p. 18–19; Myhre et al. 1977; INPFC 1981; ICCAT 1981; Orange and Calkins 1981). Because some of the catch is discarded in many fisheries, reported catch or CPUE is usually in terms of landings (Myhre et al. 1977) or nominal catches (ICNAF 1980). The procedure for estimating CPUE, however, is identical when only landings are known as long as CPUE is also in terms of landed catch. If total standardized effort is available along with total catch, then CPUE is known and need not be estimated. On the other hand, if total effort is unknown or if standardization of gear is not possible for certain elements of the fishery as in the case of the halibut fishery, then CPUE must be estimated from a sample of log books of appropriate vessels. An analogous development can be made for fisheries where total effort is known and total catch must be estimated, although we know of no examples of this type of reporting system.

For each region r, the total catch C_{rf} is assumed to be known for auxiliary variables f, which designate factors which influence effort and CPUE (e.g. nation, gear-type, and season). The use of auxiliary variables is a novel feature of this methodology, especially in the later development of statistical properties. In this section we are concerned with the statistical properties of estimators of CPUE based on sample data as a representation of the unknown CPUE in the fishery. In a later section, we explore the statistical properties of estimators of CPUE as a representation of the density of the fish population.

The sampling unit depends on the fishery and may be a vessel-day, vessel-trip, etc. From a total of V sampling units, catch (C') and effort (E') statistics are collected from V' sampling units and are suitably standardized (e.g. Myhre et al. 1977).

A simple ratio of means estimator

$$(1)\quad \widehat{\mathrm{CPUE}}_{rf} = \sum_{v=1}^{V'} C'_{vrf} \Big/ \sum_{v=1}^{V'} E'_{vrf}$$

is frequently used as an estimator of

$$(2)\quad \mathrm{CPUE}_{rf} = \sum_{v=1}^{V} C_{vrf} \Big/ \sum_{v=1}^{V} E_{vrf} = C_{rf}/E_{rf},$$

which requires that the variance of each catch statistic is proportional to its effort statistic (Cochran 1963; Smith 1980). Other estimators, such as a mean of ratios or a linear regression estimator, are available if different assumptions about the variance are made (Draper and Smith 1966; Smith 1980). The estimator (1) is biased, but the bias is negligible for a large sample size V'. The estimated variance of the ratio estimator $\widehat{\mathrm{CPUE}}_{rf}$ from Cochran (1963, p. 158, 163) is

$$(3)\quad \widehat{\mathrm{var}}(\widehat{\mathrm{CPUE}}_{rf}) = \frac{V'}{V'-1} \frac{1-\ell}{\left(\sum_{v=1}^{V'} E'_{vrf}\right)^2} \sum_{v=1}^{V'} (C'_{vrf} - \widehat{\mathrm{CPUE}}_{rf}\, E'_{vrf})^2$$

where $1-\ell$ is the finite population correction, $1 - V'/V$. If the total number of sampling units V is unknown, then ℓ may be estimated by the proportion of catch sampled or ignored if a small proportion of the catch is sampled.

The estimated total effort $\hat{E}_{rf}$ (Gulland 1969, p. 53) is extrapolated from known catch and sample CPUE by recasting (2) as

$$(4)\quad \hat{E}_{rf} = \frac{C_{rf}}{\widehat{\mathrm{CPUE}}_{rf}}.$$

Total catch and effort for the region are then obtained from the sums of catch and estimated total effort over the subscript f, so that the region's CPUE is estimated by

$$(5)\quad \widehat{\mathrm{CPUE}}_{r} = \sum_{f} C_{rf} \Big/ \sum_{f} \hat{E}_{rf} = C_r/\hat{E}_r.$$

Rewriting (5) as

$$(6)\quad \widehat{\mathrm{CPUE}}_{r} = \left[\sum_{f} \frac{C_{rf}}{C_r} (\widehat{\mathrm{CPUE}}_{rf})^{-1}\right]^{-1},$$

the approximate estimated variance from the delta method (Seber 1973, p. 7–9) is

$$(7)\quad \widehat{\text{var}}(\widehat{\text{CPUE}}_r) = (\widehat{\text{CPUE}}_r)^4 \sum_f \left(\frac{C_{rf}}{C_r}\right)^2 \frac{\widehat{\text{var}}(\widehat{\text{CPUE}}_{rf})}{\widehat{\text{CPUE}}_{rf}^4}.$$

The estimator $\widehat{\text{CPUE}}_r$ is not unbiased, because the random variable $\hat{E}_r$ is in the denominator of (5) and the expected value of an inverse is generally not equal to the inverse of an expected value. However, for a large sample size, $\widehat{\text{CPUE}}_{rf}$ is close to the true value CPUE_{rf}. Substituting CPUE_{rf} in (6) yields the true value CPUE_r after some simplification, which shows that $\widehat{\text{CPUE}}_r$ is close to CPUE_r for a large sample size, or in terms of expected values,

$$(8)\quad \mathcal{E}(\widehat{\text{CPUE}}_r) \approx \mathcal{E}(\text{CPUE}_r).$$

CPUE as an Index of Density

We now investigate the use of CPUE as an index of population density in the region. From Gulland (1969), the expected value of CPUE_r in relation to population density D_r is

$$(9)\quad \mathcal{E}(\text{CPUE}_r) = q\frac{N_r}{A_r} = qD_r,$$

where q is the assumed-constant catchability coefficient, N_r is population abundance, and A_r is the area occupied by the population.

In practice, CPUE from a fishery will indicate a higher density than actually occurs in the population area, because fishing effort is often concentrated on grounds where fish densities are highest (Paloheimo and Dickie 1964). Still, CPUE is a valid index if the ratio of true density to density weighted by the amount of fishing is constant (Beverton and Holt 1957).

The derivation of the variance of CPUE_r requires assumptions and specifications about the nature of the fishery and the distribution of fish in the region, because both catch and effort may need to be treated as random variables (Gavaris (1980) provides such a treatment). Rather than derive a probability model for catch and effort for a specific fishery, we use the estimated sample variance of $\widehat{\text{CPUE}}_r$ in (7) for comparing estimators. Although this estimator is lower than the total variability, only a relative measure of variability will be needed.

Combining Regions

Method of Area-Weighting

The first method of estimating CPUE for a regulatory area of R regions is based on the relationship between average population density of the regulatory area (D) and the individual regional densities (D_r), which is described by

$$(10)\quad D = N/A = \sum_r N_r/A = \sum_r A_r D_r/A = \sum_r a_r D_r,$$

where a_r is relative bottom area A_r/A. Substituting CPUE for density in (10), the method of area-weighting produces the estimator

$$(11)\quad \widehat{\text{CPUE}}_A = \sum a_r \widehat{\text{CPUE}}_r.$$

Its expected value from (8) and (9) is

$$(12)\quad \mathcal{E}(\widehat{\text{CPUE}}_A) = \sum a_r \mathcal{E}(\widehat{\text{CPUE}}_r) \approx \sum a_r \mathcal{E}(\text{CPUE}_r) = \sum a_r qD_r = qD,$$

which shows that $\widehat{\text{CPUE}}_A$ is approximately unbiased for qD. Estimated variance of $\widehat{\text{CPUE}}_A$ is

$$(13)\quad \widehat{\text{var}}(\widehat{\text{CPUE}}_A) = \sum a_r^2 \widehat{\text{var}}(\widehat{\text{CPUE}}_r),$$

where $\widehat{\text{var}}(\widehat{\text{CPUE}}_r)$ is computed from equation (7).

Method of Effort-Weighting

Frequently, a different method is used for estimating CPUE for a regulatory area. Regional catch and effort are summed over regions, resulting in

$$(14)\quad \widehat{\text{CPUE}}_E = \sum_r C_r \Big/ \sum_r \hat{E}_r = C/\hat{E},$$

which represents the combined CPUE for the regulatory area. Rewriting (14) as

$$(15)\quad \widehat{\text{CPUE}}_E = \sum \hat{E}_r \widehat{\text{CPUE}}_r/\hat{E} = \sum e_r \widehat{\text{CPUE}}_r,$$

this method weights each regional CPUE by the relative effort $e_r(\hat{E}_r/\hat{E})$ in the region (Gulland 1969). Noting the similarity between (5) and (14), the estimated variance of $\widehat{\text{CPUE}}_E$ is given by equation (7) with subscripts rf and r replaced by r and E, respectively. Using (15),

$$(16)\quad \mathcal{E}(\widehat{\text{CPUE}}_E) \approx q\sum \mathcal{E}(e_r) D_r,$$

which shows that the expected value is proportional to regional density weighted by relative effort. Thus, this estimator is biased as an index of D, with the bias given by

$$(17)\quad \mathcal{E}(\widehat{\text{CPUE}}_E) - qD \approx q\sum \mathcal{E}(e_r)(D_r - D).$$

The amount of bias depends on the differences between regional densities and average density.

Comparison of Methods

We compare the two methods of estimating CPUE for a regulatory area in terms of the statistical properties of the estimators. The coefficient of variation C.V. (the square root of the variance, divided by the estimator) is used as a measure of the lack of precision of an estimator. The bias of an estimator is a measure of the lack of accuracy of a method. The mean square error (Cochran 1963, p. 15), which is the sum of the variance and the square of the bias, is an alternative

measure of accuracy. An unbiased estimator is not necessarily better than a biased estimator, if its mean square error is higher.

Theoretically, there are conditions where each of the two methods is superior to the other. The theoretical justification of the method of area-weighting is the accuracy of its estimator as an index of average population density when the population area is unchanging. The theoretical justification of the method of effort-weighting is that CPUE estimates from regions with high fishing effort are more likely to be based on larger sample sizes. They are thus more precise than those from regions with low effort and, therefore, should be weighted accordingly. The area-weighted method under these conditions is likely to be imprecise. In addition, effort-weighting may be used when the spatial distribution of fishing changes in response to changes in the population area.

However, under certain conditions which we now derive, the two methods provide either identical estimates or estimates which show the same trend over time. Under these conditions, either method may be used.

Conditions for Identity

Estimators (11) and (14) are rewritten to be functions of time to facilitate comparisons:

$$(18)\quad \mathrm{CPUE}_{A,t} = \sum_r a_r \,\mathrm{CPUE}_{r,t}$$

$$(19)\quad \mathrm{CPUE}_{E,t} = \sum_r e_{r,t} \,\mathrm{CPUE}_{r,t}.$$

The two estimators $\mathrm{CPUE}_{A,t}$ and $\mathrm{CPUE}_{E,t}$ are identical when relative effort is equal to relative bottom area, i.e.

$$(20)\quad e_{r,t} = a_r,$$

or when CPUE is constant over regions for each year, i.e.

$$(21)\quad \mathrm{CPUE}_{r,t} = \mathrm{CPUE}_t.$$

By comparing (18) and (19) it is easy to see that if (20) is true, $\mathrm{CPUE}_{A,t} = \mathrm{CPUE}_{E,t}$.
If (21) is true, then from (18)

$$\mathrm{CPUE}_{A,t} = \left(\sum_r a_r\right) \mathrm{CPUE}_t = \mathrm{CPUE}_t$$

and from (19)

$$\mathrm{CPUE}_{E,t} = \left(\sum_r e_{r,t}\right) \mathrm{CPUE}_t = \mathrm{CPUE}_t,$$

because both a_r and $e_{r,t}$ sum to 1. These are the only two conditions when estimates from the two methods are equal, because two linear combinations of arbitrary variables are equal only if the coefficients are identical (Hoffman and Kunze 1971, p. 69–70). In mathematical terms, (20) and (21) are necessary and sufficient conditions for the equality of the two estimators.

Conditions for Similar Time Trends

We define a relative measure of CPUE,

$$(22)\quad \mathrm{CPUE}^*_{r,t} = \mathrm{CPUE}_{r,t}/\mathrm{CPUE}_{A,t}$$

so that $\sum a_r \,\mathrm{CPUE}^*_{r,t} = 1$. From (9) and (12), $\mathrm{CPUE}^*_{r,t}$ has the expected value of 1 when regional density is equal to density for the regulatory area.

The two estimators $\mathrm{CPUE}_{A,t}$ and $\mathrm{CPUE}_{E,t}$ show the same trend over time, i.e.

$$(23)\quad \mathrm{CPUE}_{A,t} = k_1 \,\mathrm{CPUE}_{E,t}, \text{ where } k_1 \text{ is a constant,}$$

if relative CPUE is inversely proportional to relative effort, i.e.

$$(24)\quad \mathrm{CPUE}^*_{r,t} = k_2/e_{r,t}, \text{ where } k_2 \text{ is a constant,}$$

or if relative CPUE and effort are both constant over time, i.e.

$$(25)\quad \mathrm{CPUE}^*_{r,t} = c_r, \text{ and } e_{r,t} = e_r.$$

First, to show that condition (24) results in (23), we rewrite (22) as

$$\mathrm{CPUE}_{r,t} = \mathrm{CPUE}^*_{r,t}\mathrm{CPUE}_{A,t} = (k_2/e_{r,t})\,\mathrm{CPUE}_{A,t},$$

or

$$(26)\quad e_{r,t}\,\mathrm{CPUE}_{r,t} = k_2\,\mathrm{CPUE}_{A,t}.$$

From (19) and (26), $\mathrm{CPUE}_{E,t}$ becomes

$$(27)\quad \mathrm{CPUE}_{E,t} = \sum_r e_{r,t}\,\mathrm{CPUE}_{r,t} = (k_2\,R)\,\mathrm{CPUE}_{A,t}$$

which shows that $\mathrm{CPUE}_{E,t}$ and $\mathrm{CPUE}_{A,t}$ have the same trend over time.

Secondly, we show that the pair of conditions (25) results in (23). If relative CPUE is constant over time, then (19) becomes

$$\mathrm{CPUE}_{E,t} = \left(\sum_r e_{r,t}\mathrm{CPUE}^*_{r,t}\right)\mathrm{CPUE}_{A,t} = \left(\sum e_{r,t}\,c_r\right) \times \mathrm{CPUE}_{A,t},$$

or

$$(28)\quad \frac{\mathrm{CPUE}_{E,t}}{\mathrm{CPUE}_{A,t}} = \sum_r e_{r,t}\,c_r.$$

For the ratio (28) to be independent of time, $e_{r,t}$ must be constant over time.

There may be other conditions which allow the two estimators to show the same trend over time, but these are the most straightforward.

Application to Pacific Halibut

Catch-and-effort statistics from the longline fishery for Pacific halibut are from samples of log books of individual

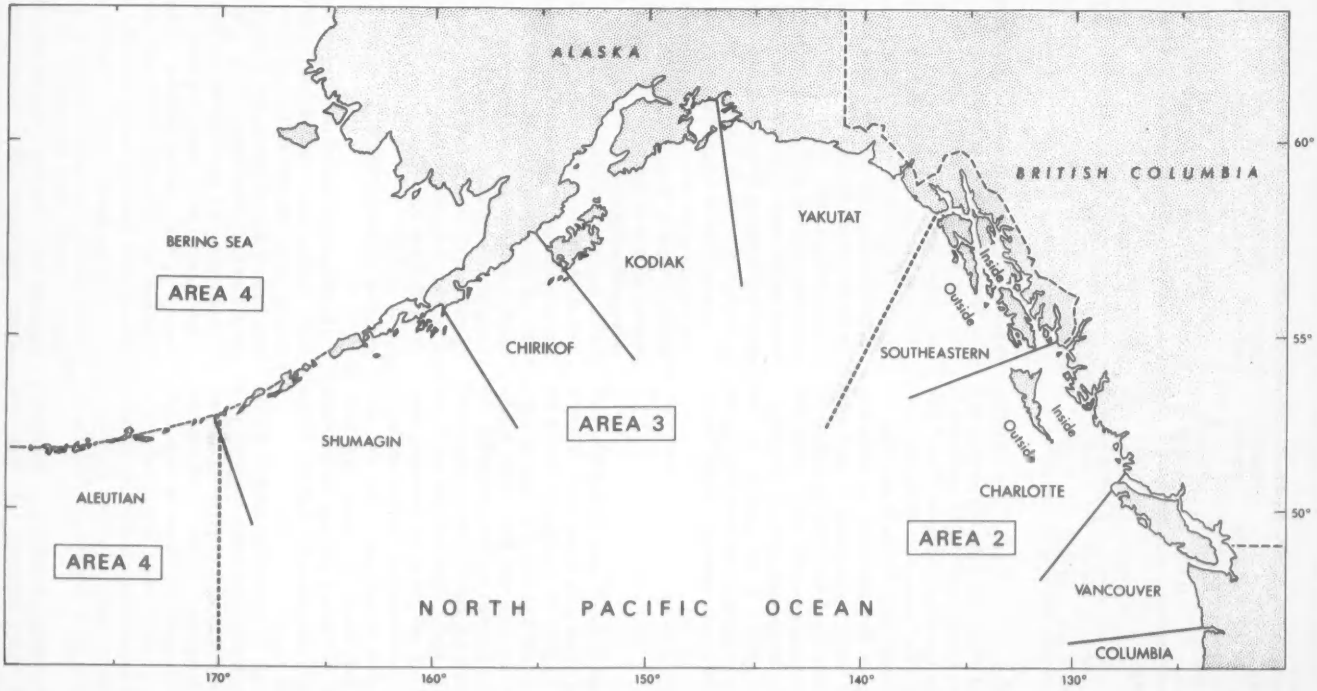

FIG. 1. Regions and current regulatory areas of the NE Pacific coast for the Pacific halibut fishery.

TABLE 1. Total bottom area (A_r) and relative bottom area (a_r) occupied by halibut in each region of regulatory areas 2 and 3.

Regulatory area 2	Vancouver	Charlotte (outside)	Charlotte (inside)	SE Alaska (outside)	SE Alaska (inside)	Total
Total area (km^2)	22 157	5862	53 256	21 385	24 061	126 721
Relative area	0.1748	0.0462	0.4203	0.1688	0.1899	1.0000

Regulatory area 3	Yakutat	Kodiak	Chirikof	Shumagin	Total
Total area (km^2)	50 605	96 291	58 751	57 694	263 341
Relative area	0.1922	0.3656	0.2231	0.2191	1.0000

fishing vessels (Myhre et al. 1977). Catch is recorded in weight of fish, and a unit of gear is a skate (100 hooks spaced 5.6 m apart along a groundline). Units of gear are standardized for differences in hook-spacing (Hamley and Skud 1978; Skud 1978). Catch-and-effort statistics are grouped by geographic regions of the northeastern Pacific Ocean (Fig. 1). The auxiliary variable in this application is nationality (Canada and the United States). Annual statistics since 1929 along with sampling procedures have been published by Myhre et al. (1977) and updated by IPHC (1979, 1980). The regions are combined into larger regulatory areas for management reasons (currently areas 2, 3, and 4 in Fig. 1). The Columbia region and area 4 generally have small catches and inadequate sampling and are not considered further in this paper.

ESTIMATES OF CPUE

CPUE and its estimated variance were calculated between 1929 and 1979 for each region using equations (1)–(7). We ignored the term ℓ in equation (3) because we viewed the catch as coming from a large population. Because the variance of CPUE generally increased with CPUE in each region, we considered the ratio estimator (1) to be applicable to the data. Sampling was meager for certain regions in some years, resulting in a large coefficient of variation. If the coefficient of variation exceeded 7.5%, the sample was considered inadequate. Because CPUEs between adjacent regions are highly correlated, a sample from an adjacent region was used instead: Charlotte–Outside was used for Vancouver in 1947, 1951, 1958, and 1962–79; Chirikof was used for Shumagin in 1935, 1939, and 1942; and Kodiak was used for Chirikof and Shumagin in 1979.

For illustrative purposes, the bottom area inhabited by halibut for each region was defined to encompass depths of 19 to 279 m, determined from planimeter tracings on United States Coast and Geodetic Survey charts (Table 1). Although the entire bottom area in a region is not fished for halibut, the bottom area should be an approximate indicator of relative area of fishing grounds among regions. Independent estimates of bottom area from cohort analysis, migration studies, and catch–effort data, which are in development, will test this assumption.

The area- and effort-weighted methods of combining CPUE were applied using equations (11) and (14), respectively. Both methods produced nearly identical estimates of CPUE over time for both regulatory areas (Fig. 2). The Spearman rank correlations are 0.99 ($P < 0.001$) in area 2 and 0.97 ($P < 0.001$) in area 3. The only major difference between the methods is in area 3 for the period 1935–1945, when the effort-weighted estimates were sometimes 5–10 kg/skate lower than the area-weighted estimates.

The effort-weighted method is more precise than the area-weighted method for both regulatory areas, although the differences are not large (Fig. 3). The C.V.'s are interpreted as relative measures of variability, because a given halibut vessel fishes several days, often at the same location. Thus, vessel days are not strictly independent, and the actual C.V.'s are probably higher than estimated.

To quantify the accuracy of each method, we estimated the mean square error, which is the estimated variance plus the estimated squared bias. Because the estimator from the method of area-weighting is approximately unbiased, the average difference between estimates from the two methods over a long period of years is an estimate of the bias of the method of effort-weighting. Generally, we found that the area-weighted method has a lower mean square error than the effort-weighted method for the halibut data, because the estimated squared bias term is larger than the estimated variance. However, this result is not conclusive, because the actual variability in CPUE is probably higher than estimated because of the lack of data independence and the treatment of catch as a non-stochastic variable.

RELATIVE CPUE AND EFFORT BY REGION

The similarity of the estimates from the two methods for the Pacific halibut data result from the conditions of similarity derived earlier. Relative CPUE and effort are plotted over time for each region in areas 2 and 3 (Fig. 4). For area 2 from 1929 to the mid-1970s, relative CPUE in the two southeastern Alaska regions and the Charlotte–Inside region fluctuated around 1, the expected value if regional CPUE is the same as average CPUE, which satisfies equation (21). For Charlotte–Outside, relative CPUE was somewhat higher than other regions, but relative effort was close to relative bottom area, satisfying equation (20). For the Vancouver region, the similarity in the two methods is due to an inverse relationship between relative effort and relative CPUE, which satisfies equation (24). Generally, relative effort is inversely correlated with relative CPUE for each region in area 2, as evidenced by Spearman rank correlations (Table 2). There has

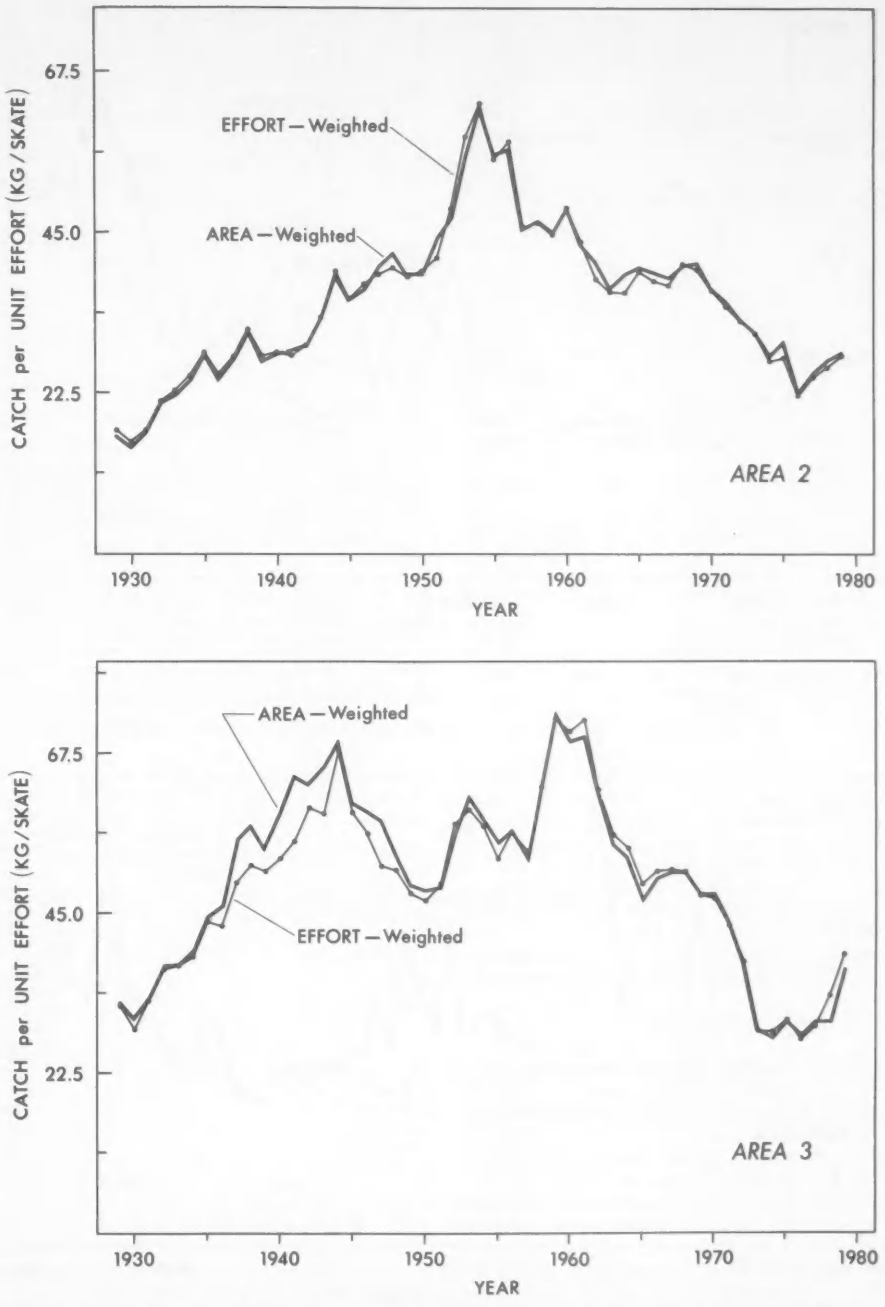

FIG. 2. CPUE estimates for areas 2 and 3 from the methods of effort-weighting and area-weighting, 1929–79.

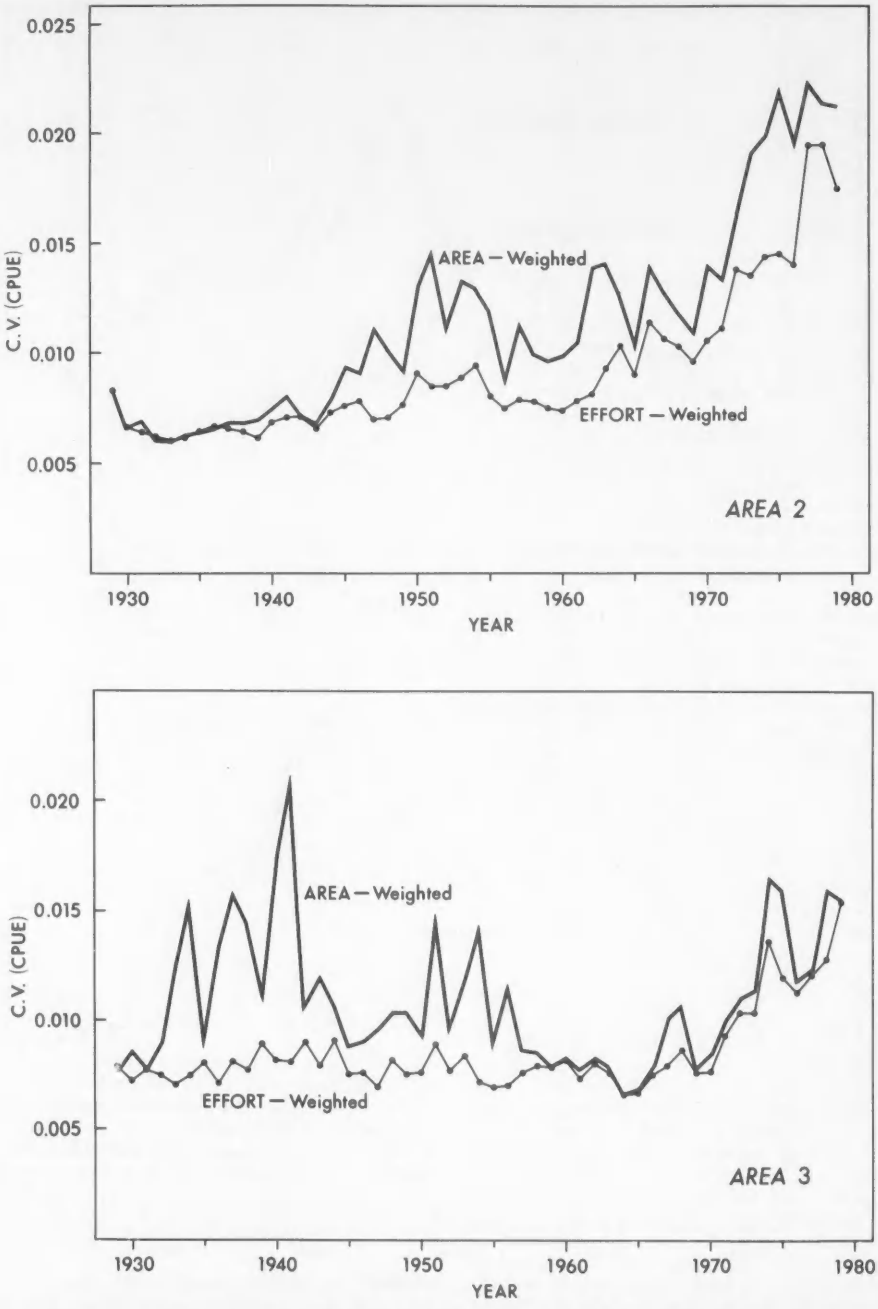

FIG. 3. Coefficients of variation of estimated CPUE for areas 2 and 3 from the methods of effort-weighting and area-weighting, 1929–79.

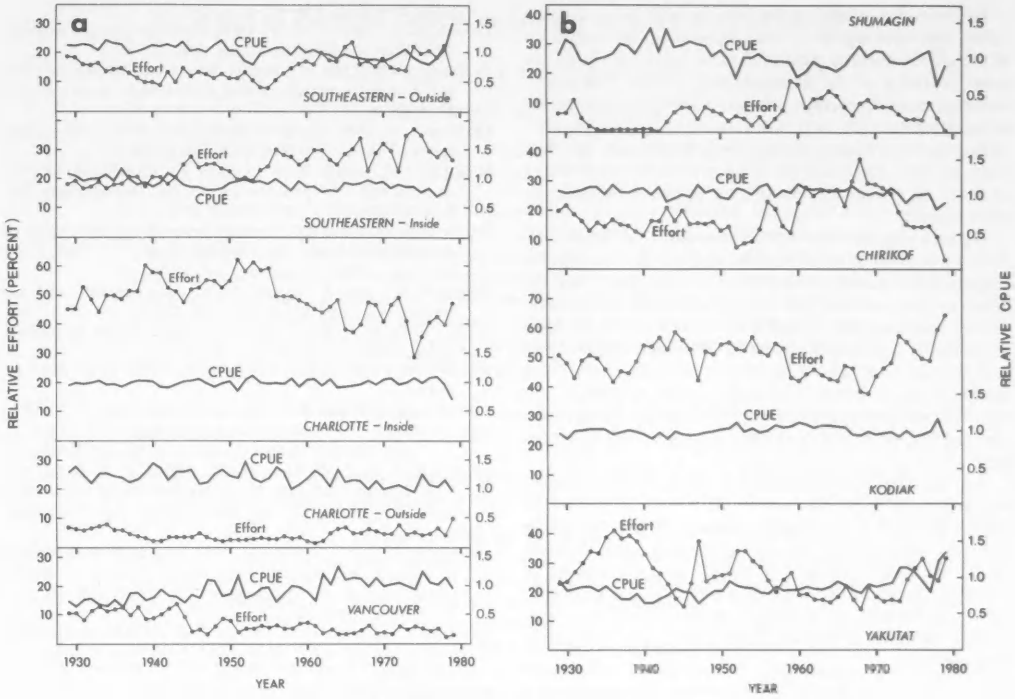

FIG. 4. Relative CPUE and relative effort for regions (a) in area 2, and (b) in area 3.

TABLE 2. Spearman rank correlations between relative effort and relative CPUE for the years 1929–79 for regions in areas 2 and 3 (ns indicates not significant, * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$).

Regulatory area 2	Correlation
Vancouver	−0.75***
Charlotte–Outside	−0.39**
Charlotte–Inside	−0.08 ns
SE AK–Inside	−0.63***
SE AK–Outside	−0.34**
Regulatory area 3	
Yakutat	−0.24 ns
Kodiak	−0.12 ns
Chirikof	0.21 ns
Shumagin	−0.25*

been a tendency in the late 1970s for relative catch and effort for regions in area 2 to show dramatic changes, which enhances the inverse relationship.

For area 3, the methods produce similar estimates, except for the period 1935–45. Relative CPUE in the Kodiak and Chirikof regions has been near 1, satisfying equation (21). Relative CPUE and effort are somewhat inversely correlated for Shumagin and Yakutat (Table 2), which satisfies equation (24). Thus, the estimates from the two methods are similar for the most part, because conditions of similarity have been satisfied. The large discrepancy in the period 1935–45 was due to relatively high effort and low CPUE in Yakutat and relatively low effort and high CPUE in Shumagin. Hence, the method of area-weighting gave more weight to the Shumagin area with higher CPUE, while the method of effort-weighting gave more weight to Yakutat with lower CPUE.

Discussion

Our development of methodology for combining catch-and-effort data requires that total catch is known for each region and auxiliary variable. If total catch must be estimated, then this source of variability can be incorporated into regional CPUE estimates using the delta method. In the case of no auxiliary variables, the area-weighted estimator can be computed without knowing total catch, and the effort-weighted estimator can be computed when only total effort is known.

We consider the methodology for estimating regional CPUE to be quite general. However, alternative estimators (Smith 1980; Gavaris 1980) can be used in lieu of (1) and (3) for estimating regional CPUE. Subsequent formulae in the development of combined estimates need not be changed.

To determine which of the two methods of combining CPUE data over regions is most appropriate, the inaccuracy of the effort-weighted method must be balanced against the lower precision of the area-weighted method. The effort-weighted method generally provides a more precise estimate because regions with large catch usually have adequte sampling, and accordingly, are weighted heavily. On the other hand, the area-weighted method is approximately unbiased as an index of population density and, hence, is theoretically more accurate.

The area-weighted method cannot be used if sufficient sampling is not obtained for all regions, or if the area occupied by the population varies and is difficult to determine. Then the effort-weighted method must frequently be used. Fortunately, the two methods produce similar results under two conditions of similarity: (1) changes in relative CPUE and relative effort over time are small among regions, or (2) relative CPUE and relative effort are inversely related over time. In addition, the two methods produce identical results: (3) if CPUE is constant over regions, or (4) if fishing effort is proportional to bottom area.

For the Pacific halibut fishery, the effort- and area-weighted methods provide similar estimates of CPUE over the 1929–79 period. In some years, relative CPUE and effort are inversely related or do not vary much over time. In other years, the fishery operates uniformly between regions in relation to bottom area, or regional CPUE is close to overall CPUE. In any case, these changes interact through the conditions of similarity to provide nearly identical estimates from both methods.

Acknowledgments

We thank D. McCaughran, R. Myhre, R. Deriso, and other members of the International Pacific Halibut Commission and V. Gallucci for their review of this paper. The referees provided helpful suggestions. We thank G. St-Pierre for doing the bottom-area tracings and E. Margeson and P. Severeid for typing the manuscript.

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An Index of Littoral Zone Complexity and Its Measurement[1]

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KENT, C., AND J. WONG. 1982. An index of littoral zone complexity and its measurement. Can. J. Fish. Aquat. Sci. 39: 847–853.

The littoral zone is important in lake ecosystems. It affects physical, chemical, and biological processes of the whole lake. We first describe patterns in the shoreline lengths of a lake drawn from maps of different scales. Second, we show that these patterns or "measurement laws" hold for many different lakes. We then use "fractal" measuring theory to provide a unifying explanation of these empirical results. Fractal theory also makes new predictions about the statistical properties of groups of lakes. Patterns in our data were consistent with these predictions. Finally, we discuss how physical and geomorphological processes can give rise to fractal patterns in the landscape.

Key words: fractal, geomorphology, littoral, morpho-edaphic index, shoreline development index

KENT, C., AND J. WONG. 1982. An index of littoral zone complexity and its measurement. Can. J. Fish. Aquat. Sci. 39: 847–853.

La zone littorale est importante dans les écosystèmes lacustres. Elle influe sur les processus physiques, chimiques et biologiques du lac entier. Nous décrivons en premier lieu la configuration de longueurs de lignes de rivage d'un lac dessinées à partir de cartes à échelles différentes. Nous démontrons en second lieu que ces configurations ou "lois de mesures" sont valables pour plusieurs lacs. Comme explication unifiant ces résultats empiriques, nous faisons appel à la théorie de la mesure des « fractures ». Cette théorie permet aussi de nouvelles prédictions quant aux propriétés statistiques de groupes de lacs. Nos données s'accordent avec ces prédictions. Finalement, nous examinons la façon dont ces processus physiques et géomorphologiques produisent des fractures dans le paysage.

Received June 8, 1981
Accepted February 15, 1982

Reçu le 8 juin 1981
Accepté le 15 février 1982

THIS paper demonstrates how two physical properties of lakes, shoreline length and littoral area, can be accurately characterized. The results are used to predict how shoreline length changes with lake area. These relationships can also predict the distribution of lakes of different size-classes. Implications for the biological processes in the lake are discussed.

Shoreline and littoral properties are known to have important predictive value in biological relationships (Rigler 1973; Barsdate et al. 1974). Simple, empirical indices of littoral extent have been used to classify diverse lakes and to evaluate the importance of their littoral areas. One of the oldest morphometric indices is the shoreline development index, defined as

$$(1) \quad D_s = L/\sqrt{4\pi A}$$

where L is the measured shoreline length, and A is the lake area. A circle gives an index of 1; larger values should indicate more convoluted figures.

The development index has been used to relate nutrient loading in a lake to the length of its shoreline (Seppanen 1972; quoted in Dillon and Rigler 1975). It has also been used in statistical studies of fish community structure (Johnson et al. 1977). However, there is a paradox associated with the calculation of the index. While the total area of the lake can be measured fairly accurately, the measurement of circumference is more arbitrary. Measured lengths are dependent on either the detail of the map or the accuracy of the measuring instrument used. Shoreline lengths that we have determined by remeasuring detailed maps may be as much as 100% greater than published values. We need to know how finer or more detailed scales of measurement change overall length estimates.

The relationship between length and scale of measurement was first explored by Richardson (1961) for curves such as coastlines. A theoretical basis for his findings was provided by Mandelbrot (1967). The surprising result of these investigations was that the measured length of the curve did not reach an asymptotic, maximum value, but continued to increase with finer and finer scales of measurement. Furthermore, the relationship between measured length and the scale of measurement was allometric. This implies that these curves do not have a well-defined length. In practice, it means that

[1]This is a contribution of the Lake Ecosystem Working Group of the University of Toronto.

Printed in Canada (J6527)
Imprimé au Canada (J6527)

TABLE 1. The latitude, longitude, and the scale of the map from which the lake outline was digitized, for the lakes used in this study.

Lake	Latitude	Longitude	Map scales used
Big Duck Lake	44°51′	78°57′	1:50 000
Bob Lake	44°55′	78°47′	1:50 000
Crotchet Lake	44°57′	78°56′	1:50 000
Gull Lake	44°51′	78°47′	1:50 000
			1:125 000
Lake Kawagama	45°18′	78°45′	1:50 000
			1:125 000
			1:500 000
Muskoka Bay	44°56′	79°24′	1:25 000
			1:500 000
Smudge Lake	44°49′	78°58′	1:50 000
Thrasher Lake	44°55′	78°59′	1:50 000

length determinations for different lakes will only be comparable if performed with the same level of detail, that is using the same scale of measurement. The length of a shoreline is then a scale-dependent quantity. This result is unsatisfying in that we cannot determine indices such as shoreline development independently of some arbitrary scale of measurement.

Mandelbrot (1977) explores the properties of highly convoluted shapes, of which river courses and coastlines are but two examples. He proposes an index which can be used to measure their degree of convolution. This index is called the fractal dimension. The fractal dimension can be determined empirically whenever there is an allometric relationship between the scale of measurement and the length measured at that scale. For a smooth curve, the fractal dimension D is 1; for more convoluted curves, D is between 1 and 2. It is determined as

$$(2) \quad D = 1 - m$$

where m is the slope of the allometric relationship:

$$(3) \quad \log[L(s)] = m \times \log[s] + a$$

where $L(s)$ is the length measured at a scale s.

Mandelbrot (1977) also predicts that lake area A and shoreline length L will be related for lakes with the same fractal dimensions D by the equation:

$$(4) \quad c_1 = L^{1/D}/\sqrt{4\pi A}; \text{ or } \ln(L) = c_2 + (D/2)\ln(A).$$

The c's are constant terms. Note that when $D > 1$, shoreline development is a biased measure of lake shoreline complexity. It will increase with lake size even for lakes with the same shape or shoreline complexity.

A third "fractal" law, known as Korcak's law, is noted by Mandelbrot (1977). If $F(A)$ gives the fraction of lakes in a region with area greater than A, lakes with fractal shoreline dimension D should satisfy the equation:

$$(5) \quad F(A) = k A^{-D/2}.$$

So there are several ways to test for fractal patterns in lake structure. We tested the applicability of the fractal measuring concept to Ontario lakes by investigating the relationship between shoreline complexity and littoral zone structure.

FIG. 1. Gull Lake as drawn by computer from digitized shoreline coordinates.

Materials and Methods

Maps at several different scales (1:25 000−1:500 000) were obtained for Canadian Shield lakes in the Muskoka and Haliburton regions of Ontario (Table 1). Lake shorelines were digitized on a Ruscom Co-ordinate Digitizer and stored as a series of x−y coordinates. The x−y coordinates fell within 0.5 mm of the original map outlines. The x−y coordinates

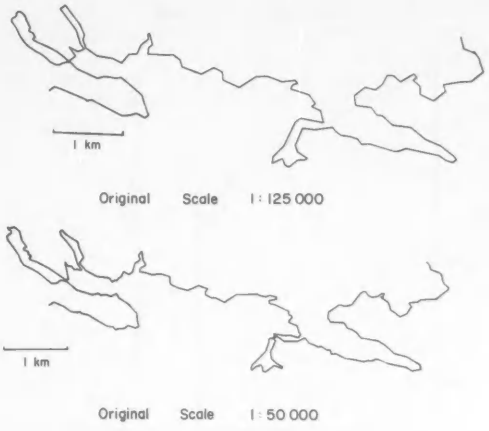

FIG. 2. Two computer-drawn outlines of a section of Lake Kawagama. Below each figure is the scale of the original map from which each shoreline was digitized.

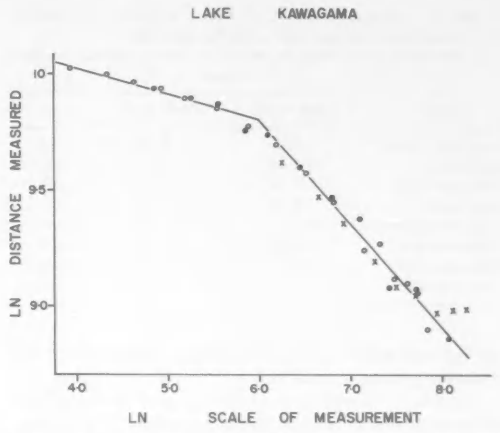

FIG. 3. The natural logarithm of the measured shoreline length (metres) is plotted against the natural logarithm of the scale of measurement (metres) for Lake Kawagama. Three different digitized representations of the same length of shoreline were produced. The x's show results from using a 1:500 000 map, the closed circles a 1:125 000 map, and the open circles a 1:50 000 map.

were joined to represent the digitized shoreline (Fig. 1). To measure a lake at a given scale s, a chain of connected line segments, each of fixed length s, was superimposed on the computer map so that: (a) the endpoints of each segment fell on the digitized shoreline; (b) shoreline path between endpoints was minimal. The measured length was then the number of line segments used times the length, s, of each segment. A correction was made to the length of the final segment so that the endpoints met. The measured length was affected by the starting point, so the procedure was replicated several times, using different starting points. This procedure is analogous to overlaying a chain with links of length s on the digitized shoreline, the ends of each link touching the shoreline, and then measuring the length of chain used.

Lake lengths were measured at scales, s, ranging from the smallest compatible with map detail to approximately one fifth of the length of the digitized shoreline. For certain lakes, several different maps were used. These maps had been drawn at scales ranging from 1:25 000 to 1:500 000. Digitized shorelines were obtained from each map. This additional information enabled us to evaluate the effect of map detail on the measuring procedure.

We tested the relationship (eqn. 4) between shoreline length and lake area by using a second data set, morphometric data on 21 lakes from the Lake Ecosystem Working Group. These lakes cover a somewhat wider region than the eight digitized lakes. As the shoreline length determinations were all taken from maps drawn at the same scale, we felt that these lengths were comparable.

To test the validity of Korcak's law, a third lake data set taken from Cox (1978) was used. Numbers of lakes with areas between 1−9 ha and 10−99 ha (Cox's size-classes V andVI) in several watersheds covering the study area (HF, HH) were used to fit equation 5.

Levels of detail on the above maps were not adequate to determine littoral zone areas, so an aerial photograph of Christie Lake (S. Ont.) with a scale of 1:7700 was digitized and measured as before. A series of zones was defined. Each zone consisted of all points within a fixed distance of shore. The areas of these zones were determined by planimetry. This constituted our fourth data set.

Results

MEASUREMENT LAWS OF SHORELINE LENGTHS

Figure 2 shows the digitized outlines of a portion of Lake Kawagama taken from maps with scales 1:125 000 and 1:50 000, respectively. Figure 3 shows the results of the measuring procedure on Lake Kawagama for three different digitized shorelines. Intuitively, one would expect that the measured length would increase as the scale of measurement decreases. This is verified by the data, as there is a significant negative correlation between the measured shoreline length and the scale of measurement ($r^2 = 0.85$). If we tentatively entertain the hypothesis that lake shorelines follow fractal laws, we can make two types of predictions. We may find that we can extrapolate from large-scale to fine-scale measurements. Secondly we may find that lakes in the same geological province are similar in their shoreline structure. They should have the same fractal dimensions.

From Fig. 3, we can see that shoreline length is not described by a simple allometric relationship. While the measurement data for the largest scale map is linear within its range, the data for both the 1:50 000 and 1:125 00 scale maps show a significant lack of fit to a single linear model. This means that we cannot extrapolate to finer scales in order to predict fine-scale shoreline lengths. However, our second prediction appears to hold as all of the lakes show a similar pattern (Table 2). This pattern involves a second linear or

TABLE 2. Large- and small-scale slopes, log breakpoint (metres) from best fitting two-line regressions for each lake.

Lake	Slopes		Breakpoint (m)
	Large scale	Small scale	
Big Duck Lake	−0.64	−0.13	255
Bob Lake	−0.44	−0.16	482
Crotchet Lake	−0.28	−0.12	178
Gull Lake	−0.27	−0.10	354
Lake Kawagama	−0.45	−0.12	354
Muskoka Bay	−0.44	−0.16	459
Smudge Lake	−0.55	−0.16	255
Thrasher Lake	−0.43	−0.16	482

fractal relationship valid at finer scales of measurement. We therefore propose a modification to our first prediction.

Suppose the shoreline of a lake is formed by at least two different geomorphological fractal processes. Each process modifies the shoreline, but at different scales. If we measure the shoreline at the scales at which the effects of only one process predominate, then we find a single fractal law. If we had two processes working at two different scales, then the resulting measurement curve would have two parts. These two line segments would be joined at the characteristic scale or breakpoint separating the two processes. If the same processes are operating on all of the lakes in our study area, then the line segments should all bend at the same scale.

To test this model, the data was partitioned into two sets, consisting of measurements at scales above or below a scale called the breakpoint. A separate line was fitted to each set. We repeated this procedure, varying the breakpoint. We recorded the breakpoint for which the regressions fit best. The results are shown in Table 2.

This analysis revealed several patterns. (A) The two-line model fit very well for each lake. (B) The slopes of the upper and lower line segments fell into two different and distinct regions. Within each region the slopes showed remarkable consistency. (C) Two processes were sufficient to explain all the measured relationships, and these processes were all separated at similar scales or breakpoints. For each lake the two-line model explains significantly more variance than the one-line model. The large-scale lines correspond to fractal dimensions clustering around a mean value ($x \pm 2$ SE) of 1.44 ± 0.09, while the dimensions at the fine scales cluster around 1.14 ± 0.02. The breakpoint has a mean value of 350 m ± 90 m.

Mapping Inaccuracies

We then checked to see if the relationships were a result of the mapping procedure used, rather than natural properties of the lakes themselves. This was why we measured Lake Kawagama shorelines from maps at three different scales. The same procedure was also applied to Gull Lake. The results for Lake Kawagama are shown on Fig. 3 and for Gull Lake on Fig. 4. Although the original scales of the maps differ greatly, there is a remarkable consistency in the results. Measurements taken from crude large-scale maps overlap the measurements taken from fine-scale maps. Therefore the results summarized in Table 2 are independent of mapping inaccuracies.

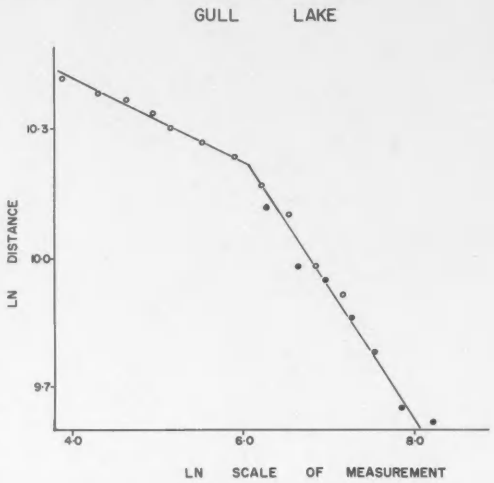

FIG. 4. The natural logarithm of the measured shoreline length (metres) is plotted against the natural logarithm of the scale of measurement (metres) for Gull Lake. Two different digitized representations of the lake were used. The closed circles show the results from using a 1:500 000 map, the open circles a 1:50 000 map.

Lake Area Versus Shoreline Length

The relation between shoreline length L (measured at a fixed scale) and lake area A was determined by a linear regression of $\ln(L)$ on $\ln(A)$. Equation 4 predicts that the slope of the fitted relationship will be half the fractal dimension D. For the 21 lakes in our second data set, the regression was highly significant; ($r^2 = 0.94$, $F_{(2,19)} = 268$, $P < 0.001$) the value of D was estimated to be 1.50 ± 0.18 (Fig. 5). This agrees with the large-scale shoreline dimension 1.44 ± 0.09 found earlier. When lakes from regions outside our study area (Parry Sound, Sudbury) were included, the estimated value of D rose to 1.59 ± 0.18 ($r^2 = 0.91$, $F_{(2,31)} = 305$, $P < 0.001$).

Distribution of Lake Areas

We also calculated the fractal dimension D from Korcak's law (eqn. 5) by using the relation:

$$(6) \quad \log_{10}\{(F(A) - F(10A))/(F(10A) - F(100A))\} = D/2.$$

When the value of A is taken to be 1 ha, the numerator and denominator above are just the numbers of lakes in size-classes V and VI in our third data set. The results are shown in Table 3. For the watersheds covering the central portion of our study area (2HF and 2HH), D was estimated to be 1.51, in agreement with our previous estimates. Other watersheds gave higher D values; the mean for five watersheds was 1.64 ± 0.11. These increased values behave like those found when equation 4 was applied to lakes from the north and west of our study area.

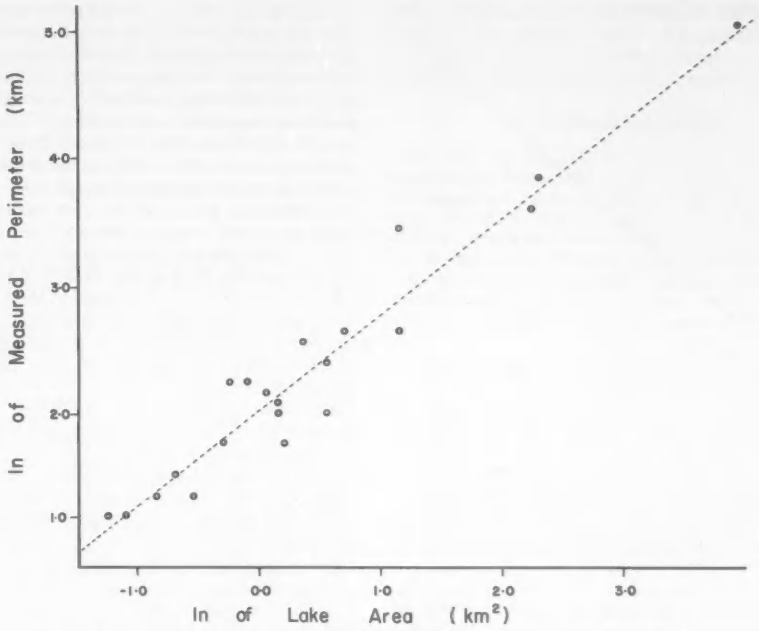

FIG. 5. The natural logarithm of lake area (km^2) is plotted against the natural logarithm of the measured perimeter of the lake (km) for 21 lakes in our study area. From the slope of this regression we are able to calculate an independent estimate of the average fractal dimension of the shoreline for lakes in this area.

TABLE 3. Areal distribution of lakes in watersheds: a test of Korcak's law.

Watershed[a]	LEWG lake group	Number of lakes in size-class V (10-99 ha)	Number of lakes in size-class VI (1−9 ha)	Estimate of D from eqn. 6
2HF+2HH	Kawartha, Muskoka	75	426	1.51
2KB+2KD	Algonquin	111	724	1.63
2DD+2CF	North Bay, Sudbury	150	1022	1.66

[a]Watersheds from Cox (1978). The correspondence between Cox's watersheds and the LEWG lake groupings is approximate.

FRACTAL SCALING OF NEARSHORE AREAS

Finally, we examined how littoral area relates to shoreline complexity. As detailed lake contour maps were not available at the scale at which we were working, we decided to test the simpler relationship of shoreline length to "nearshore" area. Specifically, we measured the area of zones within a given distance w of the shore. If the zone had smooth edges of length L then the area would approximately be equal to $L \times w$. However, we have shown that the length L depends on the scale at which the shoreline is measured according to equation 3, or setting the scale s equal to w:

$$(7) \quad L(w) = c \times w^{1-D}$$

where c is a constant and D is the fractal dimension. The area as a function of the width w of the zone would then be given by

$$(8) \quad A(w) = L(w) \times w$$
$$A(w) = c \times w^{1-D} \times w = c \times w^{2-D}.$$

For smooth curves, D is equal to 1. The constant c is then the length of the curve. The adjacent area is linearly proportional to the width of the zone. For fractal shorelines, D is greater than 1. Thus, in very convoluted lakes the shallowest parts of the littoral zone, the small bays and coves, make up a greater proportion of the zone than in less-convoluted lakes.

The heuristic argument leading to equation 8 gives a formula identical with that given by the Minkowski−Bouligand dimension law (Mandelbrot 1977). We wanted to see if these two relationships gave the same fractal dimension.

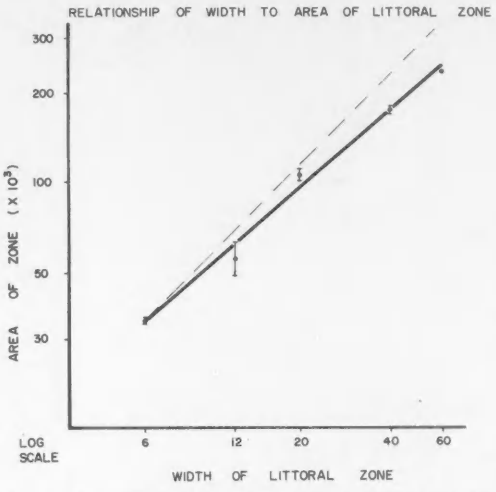

FIG. 6. The relationship between the width of the littoral zone (m) and its area (m^2) for Christie Lake. The broken line represents the expected relationship if the fractal dimension was equal to one. The open circles show the measured relationship (±2 SE).

Figure 6 shows the relationship between the width of the zone and the measured area for Christie Lake. The fractal shoreline dimension calculated from equation 5 was 1.149. When we regressed log(area) on log(width) we obtained a slope of 0.85 ($r^2 = 0.99$). This is significantly different from a slope of one ($F_{(1,26)} = 22.5, P < 0.001$). Using equation 8, we compute a fractal dimension of 1.15. Although this is but a single example, the excellent agreement between the values is highly suggestive.

Discussion

How do the fractal properties of lake shorelines contribute to our understanding of lake structure and biology? The shoreline development index is of "considerable interest because it reflects the potential for greater development of the littoral communities in proportion to the volume of the lake" (Wetzel 1977). But as Hutchinson (1957) points out, shoreline length "depends on the fineness of detail of the map and for this reason the values obtained on maps of different scale . . . may be somewhat different." Thus shoreline development "suffers from the same sources of uncertainty as measurements of length" (Hutchinson 1957). The determination of a fractal measurement law overcomes this difficulty by demonstrating a common basis for interpretation of shoreline measurements.

Lake Structure

Our data supported the hypothesis that lakes in the same region show similar fractal properties. Two fractal laws held at large and small scales separated by a breakpoint at a scale of about one third of a kilometre. How might this have come about?

Our lakes fall into Hutchinson's geomorphological type 26, in which glacial action has removed softer rock overburden and differentially deepened rock basins in zones of fracture and shatter belts (Hutchinson 1957). Ambrose (1964; quoted by Chorley 1969) suggests that ice scouring is "reactivating an ancient topography virtually intact." The fractal dimension ($D = 1.45$) found at large scales for shield lakes may reflect large-scale features, such as the distribution of fracture zones. The fractal dimension changes ($D = 1.15$) at a scale of measurement around 350 m. This should be the scale at which the glacial corrasive processes cancel each other out and erosional processes predominate. The dependence of glacial corrasion and erosional processes on preexisting geological features found over a wide area explains the consistency of the two dimensions and the breakpoint in the variety of lakes we measured. We predict that similar properties will be found for other irregular glacial scour lakes in different regions of the shield.

Three other predictions of fractal theory were tested. The shoreline length–lake area relationship of equation 4 and the lake area distribution law of equation 5 gave independent estimates of the shoreline dimension D of 1.50 and 1.51, respectively. These agree well with the large-scale D value of 1.44 found from the digitized lakes. The nearshore area relation of equation 8 independently estimated a D value of 1.15, identical with the small-scale shoreline D value. The excellent agreement of these independent dimension estimates supports the hypothesis that fractal laws govern the structure of the lakes in a region.

One consequence of the fractal structure of lakes is that the shoreline development index (eqn. 1) will be biased by lake size. If, as our data suggest, the ratio in equation 4 is constant, then shoreline development will increase with lake area. That is, if a lake were simply enlarged without changing its shape, the measured shoreline development would increase — in spite of the apparent correction for lake area which it incorporates.

In our second data set, shoreline development ranged from 3.1 to 11.7 and was significantly correlated ($P < 0.001$) with lake area. The ratio in equation 4, which represents a fractal measure of development, ranged from 0.91 to 1.28 and was uncorrelated with lake area. So fractal indices eliminate problems of scale because they are independent of measuring scale and lake size.

Community Structure

How can fractal morphometric laws help explain lake biology? We feel that fractal indices help quantify the role of the littoral zone. Most lake fish spawn in the littoral zone. The prey of many game fish are restricted to the littoral zone. In their classification of Ontario lakes, Johnson et al. (1977) attempted to explain the presence of certain fish species using limnological factors. In their analysis, the first discriminant function essentially separated deep, oligotrophic trout lakes from all others. However, the second discriminant function found the shoreline development index to be the most important of the physical factors. They interpreted this to be a measure of lake size. We suggest that this function might best be interpreted as a measure of nearshore habitat availability.

If this is true then why isn't the percent littoral area seen as

a more important physical factor? As discussed above, the shallowest portions of the littoral zone, in particular weedy bays, may be disproportionately important to many species as foraging sites. Percent littoral area may include deeper areas of less importance, especially in clear oligotrophic lakes. The fractal dimension, on the other hand, is more concerned with the complexity of the shoreline, as it emphasizes the importance of shallow waters. It allows for the separation of lake size effects from those due to nearshore habitat availability.

Littoral Productivity

A reason for the importance of littoral zones, cited by Wetzel (1975), is the fast turnover of essential nutrients where decomposers are in close proximity to the primary producers. This increases the productivity of shallow waters. The fractal relationship between the area of a given zone and its mean width could help weight the contributions of shallow and deep-water regions in proportion to their net productivity. Such a weighting may be essential for a proper understanding of larger oligotrophic lakes. In lakes with low nutrient levels, extensive littoral zones may increase productivity (Adams and Olver 1977). Johnson et al. (1977) found that oligotrophic lakes with low Morpho-edaphic indices (MEI, Ryder 1965) were more likely to contain walleye (*Stizostedion vitreum vitreum*) if the lakes were large. They proposed that "larger lakes, with low overall MEI, can have higher MEI bays." Large lakes are seen as a patchwork of regions with different MEI values. The fractal dimension of the lake could be used to predict the distribution of these regions.

One major theme in studies of lake morphology involves the view that the littoral zone is a patchy environment of varying extent, which has a disproportionate influence on the lake as a whole. The fractal properties of lake shorelines and nearshore areas may eventually allow us to predict the distribution within a lake of high MEI areas and to correct indices of productivity for shoreline effects.

Extensions and Applications of Fractal Concepts

The fractal laws of basin morphology found above should extend to the topography of entire drainage basins. Erosional processes can produce fractal landforms (Leopold and Langbein 1962; Mandelbrot 1977). An allometric relationship exists between stream order and catchment area, stream length, maximum and mean discharge, etc. (Horton 1945; Leopold and Miller 1956; Hack 1957). A fractal measurement law of river length holds for all but the largest rivers (Richardson 1961). There should be a relationship between the fractal dimension of a stream course and the fractal dimension of its watershed (Mandelbrot 1977). Therefore, in regions where erosion has shaped the landforms, lakes formed by impoundment should show fractal shoreline laws predictable from the fractal properties of nearby drainage systems.

We propose then, that in regions where previous landforms largely determine lake outline, terrestrial contour maps could be used to test fractal theory. Contour lines in valleys would be measured to determine their fractal properties. These could be compared to the area between successive contours. In this way the fractal area law could be checked when zones of constant depth are used.

Because fractal laws can predict the distribution of areas of different habitats (lakes, bays, islands), they should be important in biogeographic models. By carefully documenting the statistical properties of landform structures, a first step can be taken towards quantifying habit complexity.

Acknowledgments

We thank Ann Zimmerman, Jyri Paloheimo, Rob Kushneriuk, Roger Hansell, Michael Gates, and Nick Collins for their comments and suggestions, and Bob Bernecky for his loan of Mandelbrot's book. I.P. Sharp Associates kindly provided APL computer resources; J. Berger lent a digitizer. The authors were supported by an NSERC postgraduate fellowship and a University of Toronto Open Fellowship, as well as by NSERC operating grants to professors Paloheimo and Hansell. We also thank the Canadian Society of Zoologists for the William S. Hoar Award, given for an earlier version of this work.

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Determination of Protein Digestibility in Atlantic Cod (*Gadus morhua*) with Internal and External Indicators

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LIED, E., K. JULSHAMN, AND O. R. BRAEKKAN. 1982. Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators. Can. J. Fish. Aquat. Sci. 39: 854–861.

Chromium(III)-oxide added to feed was determined by flame atomic absorption spectrophotometry in the same Kjeldahl digest as protein ($N \times 6.25$). The recovery was 98.0% compared with only 40.0% using nitric/perchloric acid digestion. Samples of apparent indigestible protein in Atlantic cod (*Gadus morhua*) could be collected from the middle and distal part of the intestine 24 h after feeding a diet based on fillets of saithe (*Pollachius virens*). The level of reference substance and the apparent digestibility of protein indicated that feces samples from the rectum representing the feed could not be obtained until 72 h after the first feeding. Water-insoluble protein from the intestine and rectum accounted for 35–47% and 27%, respectively, of the total dry matter protein. Postdefecation loss of water-soluble nitrogenous components would therefore cause overestimation of protein digestibility. Chromium(III)-oxide and titanium(IV)-oxide were equivalent as external indicators. With titanium(IV)-oxide as indicator substance, calcium, iron, and zinc present in the feed were examined as internal reference substances. The gastric ratios of protein over calcium or zinc were compared with the corresponding ratios in the distal part of the intestine. The apparent protein digestibility agreed with the corresponding estimates obtained by titanium(IV)-oxide. Estimates of protein digestibility from capelin (*Mallotus villosus*) predating cod caught at sea using the gastrointestinal levels of calcium and zinc as internal reference substances corresponded well with estimates from experiments with cod fed whole capelin with titanium(IV)-oxide as external reference substance. The estimates indicate that samples of feces should be taken from the distal part of the intestine excluding the rectum. Because of large differences between individuals, a sufficient number of fish should be included in digestibility studies.

Key words: cod, protein digestibility, digestibility indicators, feces sampling

LIED, E., K. JULSHAMN, AND O. R. BRAEKKAN. 1982. Determination of protein digestibility in Atlantic cod (*Gadus morhua*) with internal and external indicators. Can. J. Fish. Aquat. Sci. 39: 854–861.

La spectrophotométrie d'absorption atomique de flamme appliquée au même produit de digestion par Kjeldahl que les protéines ($N \times 6{,}25$) a servi à doser l'oxyde de chrome (III) ajouté à la nourriture. La récupération est de 98,0% comparée à seulement 40,0% par digestion à l'acide nitrique/perchlorique. Nous avons prélevé des échantillons de protéines apparamment indigestes dans l'intestin moyen et postérieur de morues de l'Atlantique (*Gadus morhua*) 24 h après un repas à base de filets de lieu noir (*Pollachius virens*). Le niveau de la substance étalon et l'apparente digestibilité des protéines indiquent que des échantillons d'excréments provenant du rectum et représentatifs de la nourriture ne peuvent être obtenus avant 72 h après le premier repas. Les protéines insolubles dans l'eau et provenant de l'intestin et du rectum constituent 35 à 47% et 27% respectivement des protéines totales de la matière sèche. La perte postdéfécation d'éléments azotés solubles dans l'eau serait donc une cause de surestimation de la digestibilité des protéines. L'oxyde de chrome (III) et l'oxyde de titanium (IV) s'équivalent comme indicateurs externes. Avec ce dernier comme indicateur, nous avons examiné le calcium, le fer et le zinc présents dans la nourriture, comme substances étalons internes. Les rapports protéines:calcium et protéines:zinc dans l'estomac furent comparés avec les rapports correspondants de l'intestin postérieur. La digestibilité apparente des protéines s'accorde avec les estimations correspondantes obtenues avec l'oxyde de titanium (IV). Des estimations de digestibilité des protéines effectuées sur des morues se nourrissant de capelan (*Mallotus villosus*) et capturées en mer, avec les mêmes niveaux gastro-intestinaux de calcium et de zinc comme substances étalons internes, correspondent bien à des

Printed in Canada (J6562)
Imprimé au Canada (J6562)

estimations découlant d'expériences sur des morues nourries de capelans entiers, l'oxyde de titanium (IV) étant utilisé comme substance étalon externe. Ces résultats indiquent que les échantillons d'excréments devraient être prélevés dans l'intestin postérieur, à l'exclusion du rectum. À cause de fortes différences individuelles, les études de digestibilité devraient être menées sur un assez grand nombre de poissons.

Received June 29, 1981
Accepted February 22, 1982

Reçu le 29 juin 1981
Accepté le 22 février 1982

In both fish farming and ecological studies on productivity, it is necessary to know to what extent food is digested and food components are absorbed. Because of problems involved in measuring the feed intake and feces output, such information is difficult to obtain for fish by the conventional balance-sheet method. Information about digestibility can be obtained easier by observing, in the food and in the feces, the ratios between the food component of interest and a nonabsorbable reference substance. Several indicators are available (Kotb and Luckey 1972). In fish experiments, preference has been given to chromium(III)-oxide. Using different methods of feces sampling, experiments on protein digestibility have been carried out on rainbow trout (*Salmo gairdneri*) (Nose 1960, 1967; Inaba et al. 1962; Cho et al. 1974, 1976; Austreng 1978; Windell et al. 1978; Choubert et al. 1979), goldfish (*Carassius auratus*) (Nose 1960), and channel catfish (*Ictalurus punctatus*) (Page and Andrews 1973).

However, studies of digestibility in wild fish must be based on components, which are present in their natural food, possessing or nearly possessing the properties of an indicator substance. Buddington (1979) used this method in a study of digestive efficiency in redbelly tilapia (*Tilapia zilli*), where cellulose, present in the natural food, was assumed indigestible and was used as a reference. In warm-blooded animals, the intestinal absorption of several elements is well regulated through biochemical/physiological processes. Atlantic cod (*Gadus morhua*), which is a predator living mainly on other fish species, prawns, and euphausides, has a large intake of different elements. Preliminary analyses of gastrointestinal contents from cod showed that calcium, iron, and zinc were concentrated along the alimentary canal, suggesting that these elements are ingested much in excess of what can be absorbed.

The present study was therefore undertaken to evaluate the elements calcium, iron, and zinc as internal indicators compared with chromium(III)-oxide and titanium(IV)-oxide as external indicators. It was established to what extent calcium, iron, and zinc could be used as reference components in studies of digestibility in wild cod.

Materials and Methods

Cod, weighing between 1.0 and 3.5 kg, were obtained at different times from different localities at the western and northern coast of Norway and from the Barents Sea. They were transferred to a sheltered 25-m^3 tank supplied with running seawater at 8°C and 35‰ *S*. The photoperiod was automatically regulated to 12 h light and 12 h dark, and the fish were always acclimatized to the experimental conditions for 10 wk. Whole capelin (*Mallotus villosus*) or sprat (*Sprattus sprattus*) were fed daily ad libitum during the acclimatization. To avoid a drastic change in the environment to which the fish had been acclimatized, the tank was divided into two compartments prior to the experimental period by insertion of screening. A number of fish which fed well were selected for the experiment and isolated in one of the compartments. Imposed 5-d starvation periods were used before experimental feeding to ensure an empty alimentary canal.

Feeding Experiments

Six feeding experiments were performed. In Expt. 1 70 fish ranging from 1.1 to 1.8 kg were force-fed moist pellets, 2 cm in diameter and of a length of 4 to 8 cm, consisting of 86% minced fish fillet from saithe (*Pollachius virens*), 7% fish oil, and 7% dextrin to which 0.1% chromium(III)-oxide was added. Four centimetres of the moist pellet corresponded to 16 g of feed, and each fish was fed 16 g per kilogram body weight. Gastrointestinal content from 10 fishes was sampled at ~4, 8, 12, 24, 36, 48, and 72 h after feeding.

Experiments 2 and 3 included seven and eight cod, respectively, averaging 1.5 kg. The food consisted of capelin ground into a paste and processed into moist pellets, 2 cm in diameter by 5 cm in length. The food in Expt. 2 contained 0.1% chromium(III)-oxide, whereas the food in Expt. 3 contained 0.5% titanium(IV)-oxide. The fish were fed to saturation twice daily for 5 d.

Experiments 4 and 6 comprised groups of eight and nine cod, respectively, averaging 1.5 kg. They were fed whole sprats (Expt. 4) and whole capelins (Expt. 6) sorted out to be 16 cm long weighing on average 15 g. Because a more stable suspension was obtained with titanium(IV)-oxide than with chromium(III)-oxide, the former was considered more convenient in these experiments. Immediately before feeding 0.1 mL of a suspension of titanium(IV)-oxide was injected into the stomachs of each feed fish. The concentration of the indicator substance was 0.5%. The fish were fed ad libitum twice daily for 9 d.

Experiment 5 included 10 cod averaging 1.7 kg. The experimental feed was identical with the feed of Expt. 2. The fish were given two pellets daily for 5 d by force-feeding.

All fish were killed by a blow on the head. The abdomen was cut open and the gastrointestinal tract was divided into six segments by ligations at the esophagus, immediately before the pyloric ceca, at the first cranial, the first caudal, the second cranial bend of the intestine, at the ileorectal valve, and at the anus. Starting with the stomach, the segments were numbered from one to six, representing 12, 6, 32, 30, 12, and 8% of the total length, respectively. The content of the segments was washed into containers with 0.9% NaCl solution. The samples were frozen immediately and lyophilized. In all experiments, except Expt. 5, analyses were carried out on pooled samples prepared by mixing equal amounts of homogenized dry matter from corresponding segments. In Expt. 5,

analyses were performed on individual samples from all fish.

Finally 15 cod caught at three trawling stations on the Skolpen Bank were sampled aboard the R/V *G.O. Sars*. The fish had predated on capelin as the sole food source. The water temperature was 3–4°C. Immediately upon the catch the fish were gutted and the alimentary canal ligated as described above, removed from the fish, and frozen. The frozen samples were brought to the laboratory, thawed, and analyzed as the samples in the above described experiments.

Analytical Methods

To determine protein ($N \times 6.25$), chromium, and titanium the samples were digested by the micro-Kjeldahl technique. In the recovery experiments for chromium three replicates of 10–200 mg of sample were used. However, two subsamples of 40 mg were routinely weighed into 75-mL digestion tubes. Five millilitres of concentrated sulfuric acid was added and the tubes were heated in a thermostat-regulated heating block (Tecator). At 200°C 3.0 g of K_2SO_4 and 15 mg of selenium were added in the form of two tablets of KJELTABS AUTO. The temperature was further raised to 375°C for 1 h. After dilution to 75 mL with distilled water, aliquots were taken for analyses of nitrogen, titanium(IV)-oxide, and chromium.

Nitrogen was determined by a salicylate–nitroprusside–dichloro-isocyanurate reaction modified to an automatic analyzer system as described by Crooke and Simpson (1971). The reagents used were an aqueous solution containing 8.5% sodium–salicylate and 0.6% sodium–nitroprusside, and 0.5% sodium–dichloro-isocyanurate in 0.6 N sodium hydroxide. The emerald green color was developed for 15 min at 37°C and the absorbance read at 660 nm in a 10-mm cell. The concentration of nitrogen was calculated from a standard curve with ammonium sulfate, which was run with each determination.

Chromium, calcium, iron, and zinc were determined by atomic absorption spectrophotometry (AAS). Chromium was determined in aliquots of the Kjeldahl digest. For the determinations of calcium, iron, and zinc, three replicates of 0.1 g were digested in 10-mL capped vials (Sovirel) with 2 mL of concentrated nitric and perchloric acid (1:1, v/v, Merck Suprapur) according to Julshamn and Braekkan (1975).The samples were preashed overnight after which they were heated for 2 h at 110°C in a pressure boiler. Deionized distilled water was added and the solutions were warmed to expel nitrous gases. The final volume was brought to 10 mL with deionized distilled water. The measurements were carried out on Perkin–Elmer atomic absorption spectrophotometers, models 370 and 403. The absorption of all elements was read in a C_2H_2/air flame with single element hollow cathode lamps. The wavelengths were set according to the instrument manuals.

Titanium(IV)-oxide was determined according to Njaa (1961): 10-mL portions of Kjeldahl digest was taken for analysis; 2 drops of a 1:1 37% H_2O_2/distilled H_2O solution was added. Upon mixing an orange–yellow color was developed. The optic density was read in 30-mm cells on a Zeiss PM2 spectrophotometer against samples without added H_2O_2.

Soluble dry matter was determined in 400-mg samples suspended in 5 mL distilled water. After 10 min of soaking they were centrifuged for 50 min at 10 000 × *g* at 4°C. The residues were suspended in 5 mL of distilled water and centrifuged again, and the washings were combined with the original supernatant liquor. Soluble dry matter was determined after freeze-drying and weighing of the supernatant fractions.

The digestibility was calculated as the apparent recovery of ingested protein (ARP) according to Njaa (1961) from the nitrogen and chromium, titanium(IV)-oxide, calcium, iron, and zinc values in the feed and in the different segments of the gastrointestinal tract, as follows:

$$ARP_f = 100(S_q/I_q)$$

where I_q and S_q are the quotients, protein over indicator substance in feed and in the segment in question, respectively. When the digestibility was related to the gastric content, the quotient I_q was replaced by G_q, which is the quotient, protein over indicator substance in the stomach:

$$ARP_s = 100(S_q/G_q).$$

The relationship between apparent digestibility (ADP) and ARP is given by:

$$ADP = 100 - ARP.$$

Concentration ratios were calculated as the quotients indicator level per gram dry matter in the gastrointestinal segment in question over indicator level per gram dry matter in the feed CR_f or stomach CR_s.

Results and Discussion

The use of chromium(III)-oxide in studies of protein digestibility has required separate digestions and determinations of protein and chromium in feed and feces. Protein has been determined according to the micro-Kjeldahl technique. The determination of chromium has included a sample digestion step with concentrated nitric and perchloric acid (Furukawa and Tsukahara 1966; Petry and Rapp 1971). We eliminated this disadvantage by determining chromium in the micro-Kjeldahl digest for protein. Both the sulfuric acid and the catalyst (KJEL-TABS) interfered, however, with the absorbance when the chromium was determined by AAS (Fig. 1). When sulfuric acid was added to the standard of chromium, the curve obtained showed a substantial inhibition compared with an untreated standard. The absorbances were further suppressed when the sulfuric acid was combined with the catalytic tablets. The determination of chromium in the Kjeldahl digest of a sample of feed to which chromium was added showed no interference.

When $HNO_3/HClO_4$ was used as a digestive system the recovery was only 40% of the corresponding recovery using the Kjeldahl system, 750 ± 100 and 1840 ± 150 mg/kg dry matter, respectively. Different sample sizes affected the recovery of chromium (Fig. 2). In samples weighing from 10 to 200 mg the recovery of chromium varied from 97.5 to 107.0% with an average of 99.4%. From the standard deviation we judged the reproducibility to be better in samples weighing from 30 to 200 mg than in samples weighing less than 30 mg. The average recovery in the samples ranging from 30 to 200 mg was 98.0%.

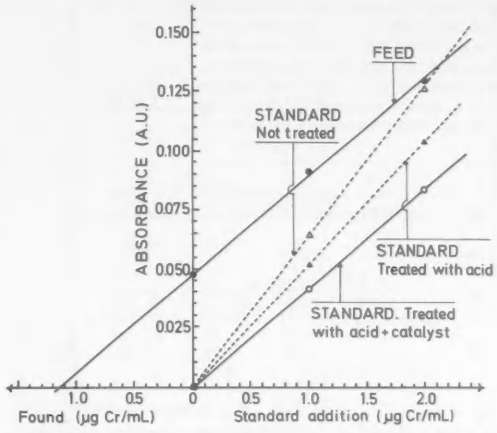

FIG. 1. Calibration curves for chromium standard in deionized water or standard treated with sulfuric acid or with sulfuric acid plus selenium catalyst. Corresponding curve for feed after Kjeldahl digestion. Absorbance given as arbitrary units (A.U.) corresponds to instrument readings.

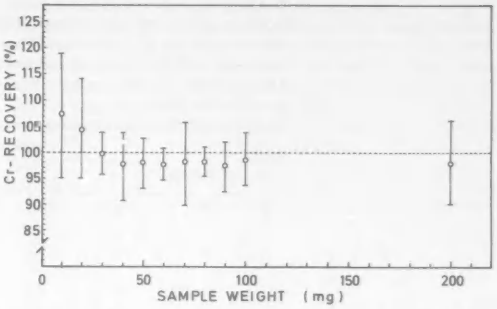

FIG. 2. Effect of sample size (dry matter) on the recovery of chromium in Kjeldahl digests. Values are means with vertical bars ±1 SD. The dotted line indicates 100% recovery of chromium added to the feed.

EXTERNAL INDICATORS AND PROTEIN DIGESTIBILITY

Njaa (1961) found titanium(IV)-oxide a useful marker in studies of protein digestion because it can be analyzed in Kjeldahl digests of feed and feces. A comparison between titanium(IV)- and chromium(III)-oxide was carried out (Expt. 2 and 3 in Tables 1 and 2). The indicator substances accumulated proportionately along the alimentary canal. The highest concentrations were found in the fifth segment, in which chromium and titanium were concentrated to 4.85 and 3.71 times the level of feed, respectively. Except for the rectum, almost identical concentration ratios were obtained in corresponding segments when calculation of ratios was related to the levels of indicator in the stomach. The digestibility of capelin protein was 83 and 82% with chromium(III)-oxide and titanium(IV)-oxide, respectively, if samples of feces were collected from segment 5 and the estimates related to the indicator level in the stomach (Table 2). A greater disagreement between chromium- and titanium-related estimates arose if calculations were related to the indicator level in the feed. The difference was, however, reduced to 2% if ADP_f in segment 5 was related to ADP_f in segment 6 in Expt. 2 and 3, respectively.

A previous digestibility experiment in cod (Tönnessen 1979) failed because of apparent loss of indicator in the posterior intestinal tract relative to the content of the feed and stomach. An explanation of these observations must be speculative. Retention of indicator substance as well as variable filling of the intestinal tract, as suggested by Edin (1926), may be possible causes of apparent loss of indicator in experimental periods.

With the exception of the stomach, the concentration of chromium(III)-oxide increased with time after feeding in all segments of the intestinal tract (Fig. 3). The gastric concentration of chromium varied between 2.39 and 2.81 mg/g dry matter and never exceeded the level in the feed, which was 2.85 mg/g dry matter. The indicator could be traced in the fifth segment 12 h after feeding. The level of chromium had, however, only been stabilized in the pyloric ceca (segment 2) with an average CR_f of 1.27.

Considerably higher levels of chromium were found in the anterior part of the intestine (segment 3) with a further increase as the chyme was transported towards the rectum. After a period of 12 h, the concentration of chromium in segment 3 had reached 6.97 mg/g dry matter with a corre-

TABLE 1. Comparisons between the gastrointestinal accumulation of chromium and titanium in cod fed minced capelin and between the gastrointestinal accumulation of calcium, iron, zinc, and titanium in cod fed whole sprat. The concentration ratios of the indicator substances are related to the level of indicator in the feed (CR_f) and in the stomach (CR_s).

Expt. no.	Indicator	CR_f; segments						CR_s; segments				
		1	2	3	4	5	6	2	3	4	5	6
2	Cr	1.32	2.05	2.74	4.69	4.85	3.05	1.55	2.07	3.54	3.67	2.30
3	Ti	1.00	1.57	2.14	3.67	3.71	3.55	1.57	2.14	3.67	3.71	3.55
4	Ca	3.57	—	5.37	8.18	9.09	4.56	—	1.49	2.29	2.54	1.27
	Fe	4.70	—	4.40	5.60	7.20	3.84	—	0.93	1.20	1.53	0.83
	Zn	2.57	—	3.77	5.96	6.31	3.36	—	1.46	2.31	2.44	1.30
	Ti	1.10	—	1.24	2.57	2.64	2.50	—	1.12	2.33	2.40	2.28

TABLE 2. Estimates of apparent protein digestibility measured in samples collected from the middle and posterior part of the intestine in cod fed minced capelin with chromium(III)-oxide and titanium(IV)-oxide as indicators, and in fish fed whole sprat with titanium(IV)-oxide as external and calcium, iron, and zinc as internal indicators. The estimates are related to the indicator and protein level in the food (ADP_f) and in the stomach (ADP_s).

Expt. no.	Indicator	ADP_f; segments			ADP_s; segments		
		4	5	6	4	5	6
2	Cr	89	90	86	81	83	76
3	Ti	89	82	88	83	82	88
4	Ca	92	93	86	72	75	49
	Fe	88	91	83	46	60	23
	Zn	89	90	81	72	75	51
	Ti	75	77	74	72	75	71

sponding CR_f of 2.45. It lasted another 10–12 h, however, before a representative digest of the feed was found in the posterior part of the intestine (segment 5). Between 24 and 48 h only slight changes of indicator levels in segments 3 and 4 could be observed. In segment 3 the concentration varied within 7.69 and 8.52 mg/g dry matter, i.e. CR_f values of 2.70 and 2.98, respectively, and in segment 4 within 8.43 and 9.68 mg/g dry matter, with corresponding CR_f values of 2.95 and 3.40, respectively. The ratio of chromium in segment 3 to chromium in segment 4 in the same period was fairly constant.

In segment 5 the level of indicator substance reached a maximum 48 h after feeding with 11.5 mg Cr/g dry matter which is equivalent to a CR_f of 4.0.

Because samples of feces in fish often are collected from the rectum (Nose 1967; Singh and Nose 1967; Page and Andrews 1973; Austreng 1978; Windell et al. 1978), it is worth noting that the concentration of indicator in the rectal content (segment 6) was still low 24 h after feeding. Chromium was concentrated from 4.40 at 24 h to 5.78 at 36 h and 9.80 mg/g dry matter at 48 h after feeding. The highest level, 12.0 mg/g dry matter, was found after a digestion time of 72 h, corresponding to a CR_f of 4.45. At this time, the stomach and pyloric ceca were empty and the levels of chromium decreasing in segments 3, 4, and 5, showing that the digestion and absorption of ingested feed had terminated.

Protein digestion and absorption seem to take place mainly in the pyloric ceca and anterior part of the intestine (Table 3). Measurements in chyme samples from the third segment at 12 h showed a digestibility of 87%, which increased to 91% at 48 h. Although small, a further increase in protein digestion and absorption could be observed at 24 h as chyme passed into segments 4 and 5 with 92 and 93%, respectively. In the period 24–48 h, there were only minor changes of coefficients in segments 4 and 5. At 72 h the decrease in chromium concentrations in segments 3 and 4 was followed by lowered coefficient of digestibility. In segment 5 the coefficient was, however, still at a level of 92%. The slow rectal accumulation of chromium(III)-oxide was reflected by the ADP_f. Only at 72 h after feeding, the digestibility had increased to 93%, which equals the levels found in segments 4 and 5 in the period 24–48 h. The rate of digestion obviously must be considered when samples of feces are collected from the rectum. The coefficients of apparent digestibility show that feces should be sampled from the intestinal segments 4 and 5 at 24–48 h when protein digestibility in cod is to be measured. Feces derived from the feed given could not be obtained from the rectum until 72 h after feeding under the experimental conditions used.

Postdefecation loss of water-soluble nitrogenous components will cause overestimation of protein digestibility. Thus Windell et al. (1978), Austreng (1978), and Smith et al. (1980) found errors of 9–10% in rainbow trout. In cod the water-insoluble protein in intestinal segments 4 and 5 and in the rectum accounted for 4.8, 6.4, and 4.5%, respectively, of the dry matter, corresponding to about 37, 47, and 27%, respectively, of the dry matter protein (Expt. 1, 36 h, Table 4). Recalculation of the apparent protein digestibility (ADP_f) discounting the water-soluble protein fraction resulted in estimates of 97 and 96% in segments 4 and 5 and 96% in the rectum. These estimates are 5–14% higher than the corresponding estimates based on the total intestinal and rectal content of protein (Table 3, 36 h).

INTERNAL INDICATORS AND PROTEIN DIGESTIBILITY

Calcium, iron, and zinc present in the feed were studied as internal indicators of protein digestibility in comparison with added titanium(IV)-oxide (Expt. 4, Tables 1 and 2). Further, a comparison of Ca as internal indicator has been compared with added chromium(III)-oxide (Expt. 5, Table 5).

Like titanium(IV)-oxide, calcium, iron, and zinc accumulated in the cod intestine (Table 1). The highest levels of elements were found in the posterior part of the intestine. When cod were fed whole sprat (Expt. 4), Ca, Fe, and Zn accumulated to 9.09, 7.20, and 6.31 times, respectively, of the level of feed in the fifth segment. The corresponding concentration ratio of titanium(IV)-oxide was 2.64. When Ca, Fe, and Zn concentrations in the fifth segment were related to the gastric content of these elements, concentration ratios of 2.54, 1.53, and 2.44 were obtained. In the case of Ca and Zn the ratio corresponds with the ratio of titanium(IV)-oxide in the segment.

An increase in element concentration in the stomach compared with the feed was observed. This relationship, which most probably was due to the drinking of seawater, led to a

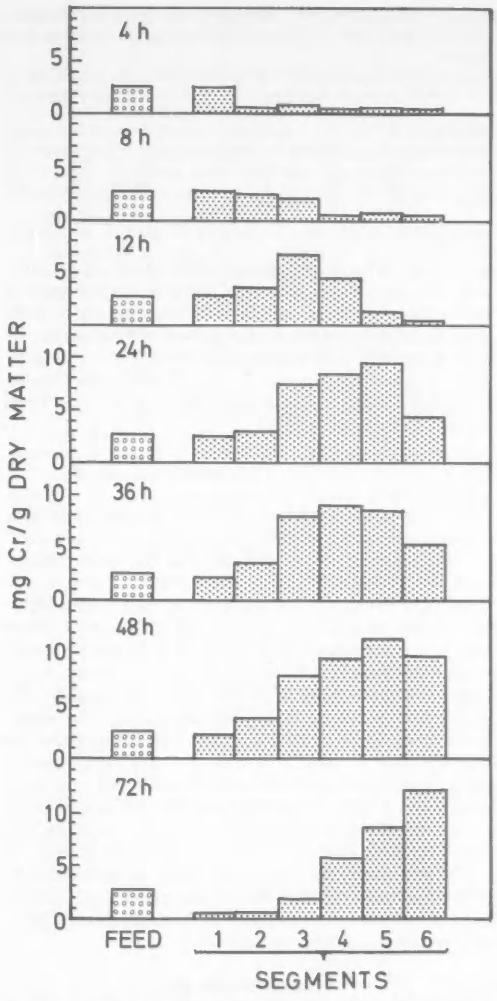

FIG. 3. Accumulation and flow of chromium(III)-oxide in the gastrointestinal tract of cod (Expt. 1). Samples were collected at different times after feeding, and the level of indicator substance is given as mg chromium per gram dry matter.

lack of correspondence between CR_f and CR_s values as well as ADP_f and ADP_s values of calcium, iron, and zinc. The differences in ADP_f and ADP_s values of titanium(IV)-oxide were small showing estimates of digestibility of 77 and 75%, respectively. Identical estimates of digestibility were obtained when the quotients protein over calcium, zinc, and titanium(IV)-oxide were related to the corresponding quotients in segment 5. This indicates that gastrointestinal levels of calcium and zinc may be used as indicators in estimating protein digestibility in cod. The gastric absorption of protein is assumed to be negligible and the gastric amounts of calcium and zinc of nonfood origin are assumed to emanate from the feed. Unlike calcium and zinc, calculations based on iron as indicator resulted in considerably lower estimates of digestibility than titanium(IV)-oxide.

TABLE 3. Estimates of apparent protein digestibility in samples collected at different time intervals in gastrointestinal segments numbered from 1 to 6 of cod fed an artificial diet based on fillet from saithe as the source of protein and with chromium(III)-oxide as indicator substance. The estimates are related to the concentration of indicator and protein in the feed. Experiment 1.

Hours after feeding	ADP_f; segments					
	1	2	3	4	5	6
4	0					
8	0	38	65			
12	6	51	87	84		
24	0	33	88	92	93	
36	31	59	90	91	91	82
48	36	70	91	92	94	90
72			59	87	92	93

TABLE 4. Water-soluble and insoluble dry matter, protein ($N \times 6.25$), and water-insoluble protein in total dry matter in intestinal samples from cod; Expt. 1, 36 h.

Component, %	Segments		
	4	5	6
Soluble dry matter	37.9	36.9	36.2
Insoluble dry matter	58.7	59.4	58.7
Protein in dry matter	13.2	13.7	16.8
Insoluble protein in dry matter	4.8	6.4	4.5

In Expt. 5 (Table 5) the comparison of calcium as an internal indicator with added chromium(III)-oxide includes a study of variation between individual fish as well as different intestinal segments. Based on chromium, the calculation of ARP_f and ARP_s values in segment 4 resulted in estimates of 18% with coefficients of variation (C.V.%) of 27.2 and 26.5, respectively. Calculations in segment 5 gave estimates of 15 and 17% with coefficients of variation of 48.6 and 25.7%, respectively. Relating the calculations to the gastric content of calcium resulted in ARP_s values of 15%, which is comparable with the chromium-based ARP_f value in segment 4 as well as the chromium-based ARP_s values of segments 4 and 5, and identical with the chromium-based ARP_f value in segment 5. The coefficients of variation of estimated recovery of protein increased, however, to 58.1 and 74.2% in segments 4 and 5. From the ARP values the apparent digestibility of feed protein (ADP) used in Expt. 5 was estimated to 85% (ADP_f) with coefficients of variation of 5.2 and 5.3%, respectively, if feces were collected from the fifth segment and chromium(III)-oxide was used as indicator substance. The corresponding ADP_s value with calcium as indicator was 85%, with a coefficient of variation of 13.6%. As in Expt. 4 erroneous estimates of ADP were obtained if the quotient protein over calcium in the feed was related to any of the quotients of the intestinal segments. Compared with the ARP and ADP values of segments 4 and 5, sampling of feces from the rectum led to reduced estimates of digestibility and

TABLE 5. The variation of ARP_f, ARP_s, ADP_f and ADP_s values with chromium and calcium as reference substances. The ARP_f and ADP_f values include 13 fish, whereas the ARP_s and ADP_s values include 10 fish due to empty stomachs in 3 fish (C.V. = coefficient of variation).

		Chromium; segments				Calcium; segments			
Coefficient		3	4	5	6	3	4	5	6
ARP_f	Mean (±SD)	35.7 (±18.6)	18.0 (±4.9)	15.0 (±7.3)	29.1 (±11.3)	7.2 (±2.4)	4.7 (±1.2)	3.1 (±1.6)	5.9 (±1.3)
	C.V. (%)	52.1	27.2	48.6	38.8	33.3	25.5	51.6	22.8
ARP_s	Mean (±SD)	36.6 (±17.8)	18.1 (±4.8)	17.1 (±4.4)	30.7 (±13.8)	30.9 (±11.8)	15.3 (±8.9)	15.5 (±11.5)	23.7 (±17.6)
	C.V. (%)	48.6	26.5	25.7	44.9	38.1	58.1	74.2	74.3
ADP_f	Mean	64.3	82.0	85.0	70.9	92.8	95.3	96.9	94.1
	C.V. (%)	29.0	6.0	5.2	15.9	2.6	1.3	1.6	1.4
ADP_s	Mean	63.4	81.9	82.9	69.3	69.1	84.7	84.5	76.3
	C.V. (%)	28.0	6.0	5.3	19.9	17.0	10.5	13.6	23.0

TABLE 6. Estimates of ADP_s in intestinal segments in cod fed whole capelin experimentally, and in wild cod using capelin as the sole food organism.

	Expt. 6; segments			Wild cod; segments		
Indicator	4	5	6	4	5	6
Ca	84	88	87	85	91	92
Zn	88	92	92	80	87	88
TiO_2	82	87	85			

increased coefficients of variation independently of the indicator used. The results show that the number of fish used in digestibility experiments as well as the intestinal site of feces sampling is important to avoid erroneous ADP values due to large differences between individuals. Preferably, samples should be taken from segment 5. Alternatively, feces samples of segments 4 and 5 may be pooled as sample sizes from segment 5 tended to be small.

PROTEIN DIGESTIBILITY IN WILD COD

By comparison, estimates of digestibility with reference to gastric concentrations of calcium and zinc were carried out in wild capelin predating cod and cod fed whole capelin (Expt. 6). Titanium(IV)-oxide was included in the experimental feed (Table 6). The estimates in the experimentally fed cod with calcium and titanium oxide as indicators agreed within 2% in segments 4 and 6 within 1% in segment 5. Using the quotients protein over calcium, zinc, and titanium in segment 5, the digestibility of protein from whole capelin was estimated to be 88, 92, and 87%, respectively. The corresponding estimates in wild cod with calcium and zinc as indicators were 91 and 87%. The difference in water temperature to which the wild and experimentally fed cod were adapted was 4–5°C. Even if the rate of digestion is affected by the acclimation temperature (Tyler 1970), it seems not very likely that a difference in the acclimation temperature of 4–5°C will lead to significant differences in protein digestibility. The results therefore indicate that gastric and intestinal levels of calcium and zinc may be used as indicators to determine protein digestibility in wild cod.

Acknowledgments

We thank Mr O. M. Smestad for collecting samples from wild cod and Mr R. Solbakken for technical assistance. This research was supported by the Norwegian Fisheries Research Council grant I 711.10.

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Piscivority by Stocked Brown Trout (*Salmo trutta*) and Its Impact on the Nongame Fish Community of Bottom Creek, Virginia

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GARMAN, G. C., AND L. A. NIELSEN. 1982. Piscivority by stocked brown trout (*Salmo trutta*) and its impact on the nongame fish community of Bottom Creek, Virginia. Can. J. Fish. Aquat. Sci. 39: 862–869.

The magnitude of fish consumption by brown trout (*Salmo trutta*) was assessed by experimentally stocking large (>280 mm) and small (<280 mm) brown trout in separate sections of a Virginia stream. Large brown trout ate five species of fish consistently during the May–November, 1979, experimental period; by November, every trout stomach examined contained fish remains. Small trout rarely ate fish. Effects of fish consumption were apparent in trout growth and in abundance of prey species. Large trout grew 9.4% in length and 21.3% in weight, whereas small trout grew 4.5% in length and 4.7% in weight. Abundance of the major prey species, torrent sucker (*Moxostoma rhothoecum*), decreased between April and October in the stream section containing large trout, but remained constant in a comparable reference section; estimates of total consumption of torrent suckers by brown trout accounted for a large portion of the decrease in abundance. Predation on torrent suckers was selective for smaller individuals. The field experiment indicates that stocking large-sized brown trout may enhance growth and survival of brown trout, but that standing crops of nongame species are likely to decline.

Key words: brown trout, nongame fishes, predation, stocking strategy, stream ecology

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Dans le but de mesurer l'importance des poissons dans le régime alimentaire de la truite brune (*Salmo trutta*), des grands (>280 mm) et des petits (<280 mm) sujets ont été maintenus dans les sections séparées d'un cours d'eau de Virginie. Durant la période expérimentale de mai à novembre 1979, les grandes truites se nourrirent régulierement de cinq espèces de poissons; en novembre, les estomacs examinés contenaient tous des restes de poissons. Les petites truites mangèrent rarement des poissons. Les effets de la consommation de poissons furent évidents dans la croissance des truites et l'abondance des espèces proies. La longueur des grandes truites augmenta de 9,4 % et leur poids de 21,3 %. Chez les petites truites, ces pourcentages furent de 4,5 et 4,7 % respectivement. Dans la section du cours d'eau contenant les grandes truites, l'abondance de la principale espèce proie, le suceur torrenticole (*Moxostoma rhothoecum*), diminua entre avril et octobre, alors qu'elle demeura soutenue dans une section témoin comparable; cette diminution d'abondance de l'espèce proie s'explique par les estimations de consommation totale du suceur par la truite brune. Cette dernière s'attaque sélectivement aux suceurs de petites taille. L'expérience sur le terrain indique que l'ensemencement de truites brunes de grande taille peut favoriser la croissance et la survie de ces dernières, mais que la biomasse des espèces non recherchees par les sportifs diminuera probablement.

Received June 15, 1981
Accepted March 5, 1982

Reçu le 15 juin 1981
Accepté le 5 mars 1982

THE stocking of catchable-size trout, a major part of many fisheries management programs, may create severe interaction between large stocked trout and native fishes. Predation by large, hatchery-reared trout has been shown to increase mortality of juvenile salmonids (Ricker 1941; Alexander 1977, 1979), and it is probable that similar effects would continue throughout the life span of many nongame species that remain relatively small. Such effects would be greatest in communities without native predators (Larkin 1979), the typical situation in which catchable-size trout are

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Printed in Canada (J6539)
Imprimé au Canada (J6539)

stocked. Other interactions could affect natural mortality, reproductive potential, recruitment, and community structure, perhaps leading to reduced species richness (Johaness and Larkin 1961; Nilsson 1961).

Several factors indicate that interactions with nongame fish will be most severe if brown trout (*Salmo trutta*) are introduced. First, brown trout are less vulnerable to angling than other trout species (Cooper 1959; Shetter and Alexander 1970), therefore remaining in streams throughout the angling season. Second, winter mortality of brown trout is often lower than for other trout species (Lemmien 1957; Kelley 1965), possibly because brown trout feed throughout the winter (Brynildson et al. 1963; Millard and MacCrimmon 1972). Third, because brown trout adapt quickly to natural foods (Gordon 1970; Jenkins 1971) and are highly aggressive (Jones 1978), the species often supplants other salmonids. Fourth, brown trout are more piscivorous than other commonly stocked salmonids (O'Donnall and Churchill 1960; Alexander 1977) and become piscivorous at relatively small sizes; fish often comprise 80% or more of the consumption by brown trout longer than 280 mm (McCraig 1960; Hannuksela 1969; Alexander 1977; Wiley 1978).

We performed this study to determine how predation by stocked brown trout affects nongame fish populations and how such predation may be useful in management of stocked brown trout. We evaluated these interactions by experimentally stocking large and small brown trout in a small stream during May–November, 1979.

Study Area

The study was conducted in Bottom Creek, a 21.1-km tributary of the Roanoke River, in the Blue Ridge Mountains of southwestern Virginia (80°10′W, 37°08′N). The stream drains a 47.7-km^2 area of predominantly forested and agricultural lands. Low alkalinity (≤8 mg/L), high dissolved oxygen (≥8.9 mg/L), moderate temperatures (≤17°C), and high pH (7.5–8.0) characterize the water quality. Within the study area, average width is 8 m and average depth is 1.1 m. A 3.6% gradient maintains an erosional substrate predominately in the boulder to cobble-size range. At several locations, falls act as barriers to upstream fish movement. The study area included 675 m of stream located 2.4 km upstream of the confluence of Bottom and Goose creeks.

Previous sampling by the landowner and management biologists showed that Bottom Creek contained no salmonids, although small populations of native brook trout (*Salvelinus fontinalis*) existed in a few upstream tributaries where water temperature remained low throughout the year. Throughout this study, no salmonids other than experimental fish were observed in Bottom Creek. The fish fauna comprised 17 nongame species including cottids, cyprinids, percids, and ictalurids; common species were torrent sucker (*Moxostoma rhothoecum*), central stoneroller (*Campostoma anomalum*), bluehead chub (*Nocomis leptocephalus*), and rosyside dace (*Clinostomus funduloides*).

Methods

The study area was divided into three adjacent sections, separated by barriers to fish movement. Sections were chosen such that relative habitat types, physical and chemical factors, fish community composition, and fish abundance were similar in each (see Garman 1980).

Section A, the lowermost 300 m, had an upstream barrier constructed of 2.5 × 3.7-cm mesh welded-wire fencing supported by 4-m metal stakes driven into the substrate. Mesh size prohibited movements of stocked trout, but permitted movements of nongame fish. The 275 m directly upstream from the artificial barrier was designated section B. The upstream boundary was a series of natural falls resulting in a 4-m drop within a 20-m length of stream. The falls prevented upstream movement of stocked brown trout until November when two mature trout were collected upstream of the barrier. Because six of the seven monthly samples were collected before this time, impact on results is considered minimal. Section C, a 100-m stretch immediately upstream of section B, was used as a reference area for monitoring seasonal changes in nongame fish abundance.

Section A was stocked with "large" brown trout, 300–305 mm in total length, from Greenspring Hatchery, Bangor, Pennsylvania. All fish were age III. On May 2, 150 large trout were stocked at an initial standing stock of 12.5 g/m^2. Because section A had no downstream barrier, trout density was expected to decline over the season as some fish moved downstream.

Section B was stocked with "small" brown trout, 200–250 mm in total length, from the Marion Hatchery of the Virginia Commission of Game and Inland Fisheries. Fish were age I and II. On May 5, 100 small trout were stocked at a standing stock of 8.8 g/m^2. Additional small brown trout from the same source were held in an isolated pool of a nearby tributary for replacement of small trout that were sacrificed during the study. They were fed a pelleted diet at a maintenance ration of 1.0–1.5% body weight per day as recommended by Phillips and Brockway (1956). Replacement trout were stocked uniformly in section B after each monthly sample.

Initial standing crops of trout in section A were ~40% higher than in section B for two reasons. First, because section A had no downstream barrier, we assumed that stocked trout would disperse over a larger area than the 300-m experimental section. Electrofishing in the stream below section A indicated that some trout had moved downstream, but comparison with catch rates within section A suggested that dispersion was minor. Second, trout in section A were sacrificed throughout the experiment without replacement because additional large trout were not available. Presuming that no trout left the study section, the density of large brown trout would equal the number stocked less the total number removed in previous sampling. On this basis, density in November was 55% of stocking density (see Table 2) and average density for the experiment was 78% of stocking density, or 9.8 g/m^2. Therefore, allowing for some loss due to movements, average trout densities in sections A and B probably were nearly identical.

Trout in sections A and B were sampled monthly from the last week of May through November. Electrofishing began at dawn (05:30–06:30) and continued for ~3 h, or until 10 large trout and 13 small trout had been collected. Weight and total length were recorded for each trout collected. Stomach contents were removed by dissection and preserved in For-

malin. Ingested fish were counted, measured when possible, and identified to species by color, shape, comparison with representative scale photomicrographs, and comparative osteology (Gregory 1959). About 94% of all ingested fish were identified in this manner.

Absolute abundance of nongame fishes was estimated for all sections in April and October using the 3-run depletion method (Zippin 1958). For each estimate, a randomly selected 25-m length of stream was blocked at both ends with 0.5-cm mesh nets and sampled three times with a gasoline-powered electroshocker operating at 115 V, D.C. Relative abundance of nongame fish also was estimated monthly for each section from the catch per unit of effort in a single electrofishing sample through a randomly selected 25-m length of stream. Total length of each fish was recorded.

Calculation of Consumption Rates

Estimates of daily fish consumption (ration) by trout were made using a modification of Bajkov's (1935) formula, as described by Eggers (1979). Average number of fish consumed per trout in 24 h ($\overline{C}_{24}$) was calculated as

$$\overline{C}_{24} = \frac{(\overline{S})\ (24)\ (R)}{2.303}$$

where $\overline{S}$ is the mean number of ingested fish observed per sampled trout and R is the exponential rate of gastric evacuation.

The exponential rate of gastric evacuation (R) is expressed as

$$R = (\ln W_0 - \ln W_t)/t$$

where W_0 is the initial amount of food in the stomach at $t = 0$ and W_t is the amount at time t (Elliott 1972). Values of R were estimated as the slope of the regression line of $\ln W_t$ versus t for four temperature ranges (see Garman 1980). Because no published data exist for gastric evacuation of fish by trout, data for walleye (*Stizostedion vitreum vitreum*) (Hofmann 1969) were used in the regression. Because Swenson and Smith (1973) showed that force-feeding, as used by Hofmann (1969), resulted in a twofold decrease in walleye digestion rates, Hofmann's evacuation times were halved. Values of R were 0.025 for 4.4–6.6°C, 0.031 for 7.2–9.4°C. 0.047 for 10.0–12.2°C, and 0.064 for 12.7–15.0°C. Similarity in digestive rates of percids and salmonids could not be conclusively verified; however, Persson (1979) showed that R values for perch (*Perca fluviatilis*) and brown trout feeding on invertebrates agreed closely except that rates for trout were higher at low temperatures. Therefore, the values used here probably underestimate both R and daily ration.

Estimates of total monthly consumption of fish by the experimental trout were calculated by expanding the appropriate $\overline{C}_{24}$ estimate, based on observed stomach contents and average temperature for the month, the number of days in the month, and the number of trout in the population. Estimates of trout abundance were based on numbers stocked, numbers removed by sampling, and an assumed 20% initial loss because of emigration and natural mortality, after Shetter (1968) and Mense (1975).

Results

Consumption of Fish by Trout

Both large and small brown trout consumed fish, but consumption by small trout was negligible. No fish were found in small brown trout stomachs in four of the seven monthly samples, and only 6 of the 92 small brown trout sampled had consumed fish. Because fish were not an important dietary item for small brown trout, only data for large trout have been used for the consumption analyses that follow.

Frequency of occurrence of fish in large brown trout stomachs increased through time (Table 1). Frequency of occurrence averaged 28% for the first four monthly samples, May–August. Frequency increased to 50% in September, and to 100% in October and November. The increase was related to the ingestion of age 0 fish, as the percentage of trout with age I and older fish in stomachs was similar for the May–August (23%) and September–November (25%) periods.

The estimated average daily consumption (C_{24}) of fish by large brown trout (Table 1) followed a similar pattern as frequency of occurrence. Daily consumption by large trout remained low through July, increased from August through October, and decreased in November. Lower average water temperature in November (5°C) reduced estimated evacuation rate and daily consumption despite frequency of fish in stomachs identical with that for October, when average water temperature was 11°C.

Large brown trout consumed an estimated 5254 fish in section A from May to November (Table 1). Estimates computed in the same way for small brown trout indicated that small brown trout consumed 334 fish during the period, or 6.4% of the consumption by large brown trout.

Differences in rate of fish consumption coincide with observed differences in growth of large and small brown trout over the course of the experiment. Mean total length of large brown trout increased 9%, from 302 mm at stocking to 330 mm in November, whereas mean length of small brown trout increased 5.0%, from 262 mm to 275 mm. Mean weight of large brown trout increased 21%, from 303 to 368 g, whereas mean weight of small brown trout increased 4.6%, from 195 to 204 g. Mean weight of a large trout increased between each pair of monthly samples, but mean weight of small trout fluctuated, declining over three of seven monthly periods.

Prey Selection by Trout

Selection for prey species by brown trout was assessed qualitatively by comparison of species composition in the diet and in the stream (Table 2). Torrent suckers comprised 55% of ingested prey. Four other species — stoneroller, rosyside dace, fantail darter (*Etheostoma flabellare*), and mottled sculpin (*Cottus bairdi*) — were also represented in the diet in lower proportions. The group of three most common species (torrent sucker, stoneroller, and rosyside dace) was consumed in proportion to combined abundance, comprising 78% of the diet and 77% of the fish present in section A. When the abundance of fish in the stream is adjusted to include only fish smaller than 110 mm (the observed maximum size of ingested

TABLE 1. Number of large brown trout stomachs examined, occurrence of fish in stomachs, and estimated daily and monthly consumption of fish by large brown trout in section A of Bottom Creek.

Month	Number trout sampled	Number containing fish	Number of fish in stomachs		Average daily consumption per trout	Total monthly consumption	
			Age 0	Age 1		Age 0	Age 1
May	10	3	0	3	0.143	0	514
June	11	3	0	3	0.155	0	497
July	12	2	0	2	0.119	0	348
Aug.	10	4	2	2	0.218	292	292
Sept.	8	4	3	1	0.475	888	296
Oct.	9	9	8	2	0.648	1134	284
Nov.	7	7	5	3	0.388	443	266
Total	67	32	19	15	—	2757	2497

TABLE 2. Species composition, by proportion, of nongame fish in section A during April and in the diet of large brown trout.

Species	Relative abundance		
	Section A (all fish)	Section A (fish <110 mm)	Large trout stomachs
Torrent sucker	0.43	0.38	0.55
Stoneroller	0.20	0.13	0.08
Rosyside dace	0.14	0.22	0.15
Fantail darter	0.01	0.02	0.15
Mottled sculpin	0.02	0.03	0.08
Bluehead chub	0.06	0.06	0.0
Margined madtom	0.02	0.03	0.0
Crescent shiner	0.04	0.05	0.0
Mountain redbelly dace	0.07	0.09	0.0
Total	0.99	1.01	1.01

TABLE 3. Estimates of absolute abundance of major nongame fish species, per 25 m of sections A, B, and C in Bottom Creek, Virginia (95% confidence intervals in parentheses).

Species	Section C		Section B		Section A	
	Apr.	Oct.	Apr.	Oct.	Apr.	Oct.
Torrent sucker	172 (120–220)	166 (152–261)	41 (34–54)	110 (82–144)	116 (92–143)	72 (57–94)
Black jumprock	3 (3–4)	12 (10–20)	2 (3–4)	2 (2–3)	1 (1–2)	30 (26–39)
Stoneroller	10 (9–15)	16 (13–23)	40 (28–67)	63 (49–82)	56 (51–64)	27 (26–31)
Bluehead chub	12 (12–13)	15 (14–20)	9 (9–11)	43 (43–44)	15 (12–26)	79 (51–127)
Rosyside dace	51 (50–53)	9 (9–10)	38 (36–44)	41 (41–42)	38 (37–41)	31 (27–39)
Mountain redbelly dace	3 (3–4)	3 (3–4)	12 (12–14)	6 (6–7)	19 (17–25)	17 (9–21)
Margined madtom	5 (5–6)	5 (5–6)	16 (15–17)	6 (6–7)	5 (5–6)	11 (11–12)
Crescent shiner	13 (13–14)	13 (13–14)	13 (12–14)	8 (8–9)	12 (11–16)	11 (11–12)
Mottled sculpin	—[a]	—[a]	—[a]	—[a]	7 (7–8)	6 (6–7)
Fantail darter	28 (23–41)	28 (23–41)	13 (12–15)	5 (5–9)	3 (3–4)	7 (7–10)
Total	268	253	175	259	272	288

[a]No fish captured.

prey), those three species made up 73% of the fish present, again similar to the proportions in the diet. Relative abundance of fantail darters and mottled sculpins in stomachs were 15-fold and 4-fold higher, respectively, than in stream samples.

Lengths of fish consumed by brown trout ranged from 25 to 110 mm. Thirty-eight percent of fish observed in stomachs were 25–50 mm long, corresponding to the length range of age 0 fish as determined from length frequency distribution of electrofishing samples. Because abundance estimates of age 0 fish were not made, selection for fish smaller than 50 mm could not be examined quantitatively. Size selection among age I and older torrent suckers, the most abundant prey species, was examined by comparing lengths of ingested fish by brown trout and mean lengths of fish collected in the stream on each sampling date. Of 12 torrent suckers that were removed from brown trout stomachs and were measurable, 11 were smaller than the average size of torrent suckers in the stream on the sampling date.

Nongame Populations

Estimates of absolute abundance in April and October revealed different changes in abundance among species and sections (Table 3). Abundance of species in reference section C changed little, except for increase in black jumprock (*Moxostoma cervinum*) and decrease in rosyside dace. Total abundance estimates in April and October were nearly identical.

In section B, which contained small trout, the abundance of several species changed during the experiment. Abundance of torrent suckers, bluehead chubs, and stonerollers increased, while decreases occurred for several of the less-abundant species such as margined madtom (*Noturus insignis*), crescent shiner (*Notropis cerasinus*), fantail darter, and mountain redbelly dace (*Phoxinus oreas*). Total abundance in section B increased by almost 50%.

In section A, which contained large brown trout, substantial changes in the abundance of four species occurred between April and October. Torrent suckers and stonerollers, the two most abundant species in April, declined in abundance by 30 and 51%, respectively. Bluehead chub and black jumprock, which had ranked 5th and 10th in abundance in April, ranked 1st and 3rd, respectively, by October. Abundance of margined madtom and fantail darters doubled. Total abundance in section A remained the same.

More detailed analysis of population changes were possible for the torrent sucker, the most abundant fish in the stream and in the diet of large trout. The pattern of decreasing abundance in section A was continuous and gradual, whereas abundance indices for sections B and C remained constant throughout the experiment (Fig. 1). Declines in catch per unit of effort of stonerollers and rosyside dace in section A were similar to the pattern for torrent suckers, while catch per unit of effort of species not consumed by brown trout remained stable at all three stations.

Changes in length frequency distributions of age I and older torrent suckers occurred between April and October in section A, but not in sections B or C. Length frequency distributions of torrent suckers at all three stations were similar in April (Garman 1980), but by October the distribution in section A had shifted to larger sizes (Fig. 2). The mode of sections B and C fell in the 110- to 130-mm range, but for section A was in the 130- to 150-mm range.

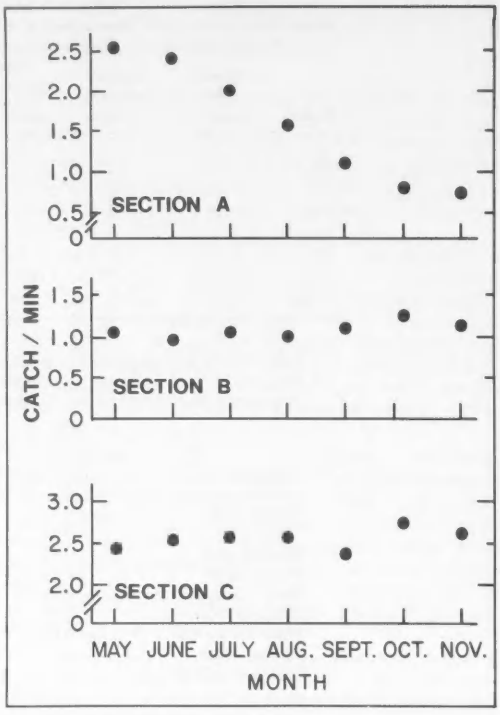

FIG. 1. Comparison of catch of torrent suckers per minute of electrofishing in sections A, B, and C, Bottom Creek, Virginia.

Discussion

The generally accepted difference in fish predation between small and large brown trout was apparent in this study. As cited in the introduction, published accounts claim that brown trout are piscivores beyond 280 mm total length. Because brown trout for the "large" and "small" treatments were larger and smaller than 280 mm, respectively, the great difference in fish consumption between treatments is empirical support for the 280-mm threshold value.

Although the field experiment was designed so that size of stocked brown trout was the only variable, logistical constraints introduced two additional variables. First, large and small brown trout came from different hatcheries and presumably from different genetic stocks. Given the high rate of transfer of trout stocks in the eastern United States and the similarity of hatchery environments among hatcheries, we believe genetic differences in the fish are of minor importance. Second, our inability to block the downstream end of section A required a higher initial stocking density of brown trout so that average density would approximate that in sec-

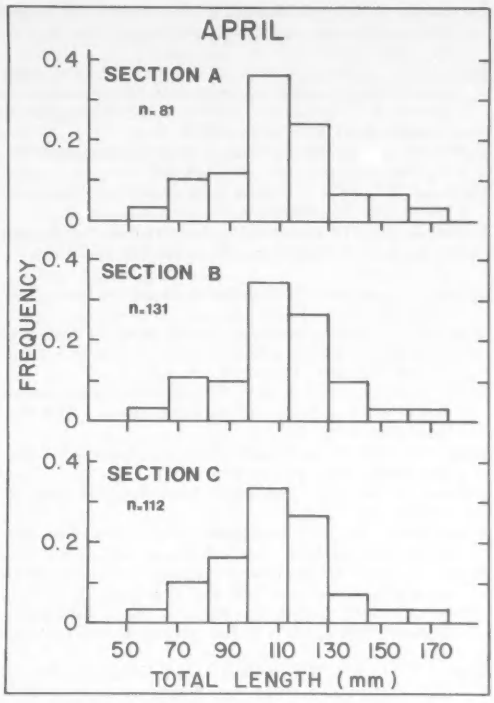

FIG. 2. Comparison of length frequency distribution of age I and older torrent suckers in October, 1979, in sections A, B, and C, Bottom Creek, Virginia.

tion B. Because standing crop of nongame fish in Bottom Creek was high, averaging 18.7 g/m^2 in April and 28 g/m^2 in October (Garman 1980), differences in predator density probably did not impact available food per predator. Furthermore, the very large difference (15-fold) in estimated total fish consumption between treatments suggests that the primary variable in the experiment, i.e. size of predator, rather than secondary variables, was the cause of the difference.

The mechanism underlying the presumed 280-mm threshold, however, cannot be identified. In this experiment, as well as in other situations (O'Donnall and Churchill 1960; Thomas 1964; Hannuksela 1969; Alexander 1977), small brown trout occasionally consumed both age 0 and older fish. Consequently, a morphological constraint on predation, such as prey girth exceeding predator mouth gape, seems unlikely. Crossman (1959) suggested that large trout may inhibit fish predation by smaller trout because of behavioural interactions. In this experiment, small trout were segregated from large trout, but still failed to consume fish regularly.

It is possible that fish prey are more vulnerable to large trout than small trout because of differences in swimming speed. Because swimming speed is a function of fish length (Beamish 1978), small trout would be less likely to catch prey fish and hence would rely more heavily on invertebrate foods. Corollary data from this study (Garman 1980) showed that stomachs of small brown trout contained ~ 10% more invertebrate food items than stomachs of large brown trout, despite the fact that the average small brown trout weighed about one-third less than the average large brown trout. The effect of differential swimming speeds also may explain selection of smaller prey by large brown trout. Smaller fish swim slower than larger fish and may lack behaviors for avoiding predators (Ginetz and Larkin 1976).

Selection for prey species is difficult to evaluate in small streams like Bottom Creek because species richness is high and absolute abundance of most species is low. Of the 17 species in the stream, only five were observed in trout stomachs. Higher proportional occurrence of fantail darters and mottled sculpin in stomachs than in stream samples may indicate selection for these demersal species by brown trout, but it is more likely that abundances of these species were underestimated in electrofishing surveys. Experience in other southwestern Virginia streams in similar watersheds indicates that these two species are frequently among the more abundant species, but are difficult to sample accurately in streams like Bottom Creek, where substrate is coarse, gradient is high, and water is turbulent. This reasoning also suggests that the 12 species not observed in trout stomachs comprise a smaller proportion of fish in the stream than the data indicate. It is probable, therefore, that the absence of these species from stomachs is an artifact of the small number of fish observed rather than negative selection against these species.

Growth differences between large and small brown trout in Bottom Creek presumably resulted from differences in diet. While many factors influence fish growth, both trout groups were exposed to similar conditions of habitat, temperature, and food resources. The only apparent difference was that large brown trout fed heavily on fish while small trout did not. The relationship between rapid salmonid growth rates and piscivority has been established previously for lakes (Reimers et al. 1955; McCraig 1960; Hiscox 1979) and large rivers (Alexander 1977). It appears that similar benefits can be expected in small streams, provided potential prey are sufficiently abundant.

Although not investigated, the continued consumption of fish by large trout during winter and spring months seems likely. Maciolek and Needham (1952) reported increases in consumption of fish by brown trout during winter in Convict Creek, California. Because condition factor and stamina play an important role in the ability of stream salmonids to survive winter conditions (Reimers 1963; Bjornn and Mallett 1964), and consumption of fish by brown trout improves condition (Reimers et al. 1955), stocking large trout could lead to increased winter survival in streams where survival of small brown trout is low. Conversely, establishment or reestablishment of forage fish populations may be beneficial in streams where growth and survival of large brown trout have been poor or where catch-and-release fisheries for brown trout are planned.

Encouraging trout predation on fish, however, may negatively affect populations of nongame species. In section A, prey species generally declined in absolute and relative abundance, and average lengths of prey species increased. Three lines of evidence support the premise that predation by brown trout was largely responsible for the reduced abundance of individual species in section A. First, large decreases in abun-

dance occurred among those species most frequently eaten by trout. Calculations based on the total number of age I and older prey eaten (Table 1) and the species composition of ingested prey items (Table 2) indicates that large brown trout consumed about 114 torrent suckers, 17 stonerollers, and 31 rosyside dace per 25-metre length of section A. This estimated consumption exceeds the absolute decrease in numbers of torrent suckers and rosyside dace and equals 50% of the decrease of stonerollers (Table 3). Second, the gradual decline in abundance of torrent suckers in section A corresponds to the continuous consumption of age I and older fish by trout in each month. Third, the change in length frequency distribution of torrent suckers occurred just above the maximum observed size of prey ingested by brown trout, and no changes occurred in sections B and C, where predation was low or nonexistent. Thus predation on fish less than 110 mm long reduced the relative abundance of fish smaller than 110 mm and those that grew into the 110- to 130-mm range by October. Because only a short stream section contained predatory trout, the observed effects on nongame species were probably mitigated by movement of individuals of affected species or sizes into the experimental section.

In streams where brown trout establish reproducing populations or overwinter survival is substantial, effects of predation on the nongame fish community may be somewhat different than those found in this study. A reduction in overall biomass of the nongame fish community and a corresponding increase in production are the most likely outcomes of sustained predation. In simple predator–prey systems involving an introduced predator, extirpation of a prey species may result (Johaness and Larkin 1961; Macan 1966; Nilsson 1972), but in more complex systems increased diversity may accompany increased predation (Clady and Nielsen 1978). Because brown trout consumed fish approximately in proportion to abundance, it is likely that predation would cause relatively equal percentage reductions in the biomass of all species rather than elimination of one or more dominant prey species.

In this regard, the potential impact of brown trout predation on endangered or threatened fishes is uncertain. Because such species usually comprise a very small proportion of the fish community, annual consumption by brown trout would be low, in both numbers and as a percentage of the total diet. Unlike other species, however, populations of rare fishes may be permanently affected by the loss of even a few individuals. Therefore, introduction of brown trout in areas containing species of special interest should be carefully evaluated.

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Biochemical and Serological Characteristics of Ontario Isolates of *Yersinia ruckeri*

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Stevenson, R. M. W., and J. G. Daly. 1982. Biochemical and serological characteristics of Ontario isolates of *Yersinia ruckeri*. Can. J. Fish. Aquat. Sci. 39: 870–876.

During testing hatchery and wild fish from Ontario sources, we isolated bacteria that had sufficient characteristics in common with *Yersinia ruckeri* to suggest they were strains of this organism. Three isolates from brook trout (*Salvelinus fontinalis*), rainbow trout (*Salmo gairdneri*), and cisco (*Coregonus artedii*) biochemically resembled the sorbitol-fermenting Serovar II strain of *Y. ruckeri*, but gave cross reactions with antiserum to the Hagerman strain, Serovar I. A fourth isolate from brown trout (*Salmo trutta*) appeared to be different from the three previously described serovars. A fifth isolate previously obtained from muskrat (*Ondatra zibethica*) intestine biochemically and serologically corresponded to the Hagerman serovar. We suggest that the geographic range of *Y. ruckeri* and variations in its biochemical and serological properties are greater than previously believed. These findings are of significance in diagnosis and regulation of enteric redmouth disease of salmonids.

Key words: *Yersinia ruckeri*, enteric redmouth, ERM, bacterial disease, fish health protection

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Au cours d'essais sur des poissons d'élevage et des poissons sauvages de l'Ontario, nous avons isolé des bactéries possédant suffisamment de caractéristiques en commun avec *Yersinia ruckeri* pour nous porter à croire qu'il s'agissait de lignées de cet organisme. Trois isolats d'omble de fontaine (*Salvelinus fontinalis*), de truite arc-en-ciel (*Salmo gairdneri*) et de cisco (*Coregonus artedii*) ressemblent biochimiquement à la lignée Serovar II, fermantant le sorbitol, de *Y. ruckeri*, mais donnent des réactions croisées avec antisérum à la lignée Hagerman, Serovar I. Un quatrième isolat de truite brune (*Salmo trutta*) semble différer des trois serovars décrits antérieurement. Un cinquième isolat, obtenu auparavant de l'intestin du rat musqué (*Ondatra ziebethica*) correspond biochimiquement et sérologiquement au serovar Hagerman. Nous suggérons que l'aire géographique de *Y. ruckeri* et les variations de ses propriétés biochimiques et sérologiques sont plus étendues qu'on ne le croyait. Ces constations sont importantes dans la diagnose et le contrôle de la maladie entérique de la bouche rouge des salmonidés.

Received October 9,1981
Accepted February 10, 1982

Reçu le 9 octobre 1981
Accepté le 10 février 1982

The enteric redmouth disease bacterium, *Yersinia ruckeri*, has been recognized as a major problem in salmonid fish farming operations in Idaho (Ross et al. 1966), and imports of carrier fish have been implicated as the probable source of disease incidents occurring outside this geographic area (Wobeser 1973). Careful screening procedures, combined with radical control measures when required, have been used to protect the Great Lakes basin fisheries from enteric redmouth disease. In 1980, for example, fingerling brown trout (*Salmo trutta*) that had been brought into Ontario were destroyed when brood stocks at the Montana source gave evidence of being carriers of *Y. ruckeri*.

Bullock et al. (1978) suggested, however, that enteric redmouth disease may have occurred in geographic areas, such as West Virginia or Australia, with no evidence of a link to imported fish. A greater base of information about the incidence of the organism and the disease is required to determine the significance, and hence, the appropriate response, when *Y. ruckeri* is detected in a group of fish. We therefore intensified surveillance of routinely tested hatchery and wild fish stocks in Ontario, to determine if there was a natural level of *Y. ruckeri* present, either as a pathogen or in a carrier state.

In the course of these studies, it became apparent that considerable uncertainty is left by the procedures for identification of *Y. ruckeri* prescribed by the Canadian Fish Health Protection Regulations, Manual of Compliance, 1977 and by the American Fisheries Society Fish Health "Blue Book" (McDaniel 1979). These procedures call for primary isolation of Gram-negative rods on tryptic soy agar plates, followed by biochemical identification tests and serological confirmation. The biochemical reactions of *Y. ruckeri* closely resemble those of *Serratia*, as noted by Ewing et al. (1978). The problems presented by such resemblances and by natural variation between strains in biochemical test results (Ewing et al. 1978; O'Leary et al. 1979) are exemplified by the report of

Printed in Canada (J6661)
Imprimé au Canada (J6661)

Llewellyn (1980), in which 10 isolates associated with the salmonid blood spot disease (SBS) of brook trout (*Salvelinus fontinalis*) and Atlantic salmon (*Salmo salar*) are described as having biochemical test reactions similar to both *Y. ruckeri* and to *Serratia liquefaciens*. These SBS isolates were apparently not tested serologically against anti-*Y. ruckeri* serum. However, confirmation of potential *Y. ruckeri* isolates is made more complex by the recent recognition that at least three distinct serovars occur (Bullock et al. 1978).

We report here on five isolates of bacteria from Ontario sources with sufficient characteristics in common with *Y. ruckeri* to suggest that they are strains of this species. As such, they are of significance with regard to concepts about the geographic range of this organism and its biochemical and serological characteristics, and to fish health regulatory activities.

Materials and Methods

Isolation and Biochemical Testing

Samples of heart, liver, spleen, and kidney tissue were removed aseptically from adult fish and placed in tubes of tryptic soy broth (TSB) for enrichment. After 48 h at 20°C, enrichment broth was streaked onto tryptic soy agar (TSA) plates and incubated 24–48 h at 20°C. Colonies of Gram-negative rods giving a negative cytochrome oxidase reaction when tested with a 1% aqueous solution of *p*-aminodimethylaniline oxalate (Difco) were selected for further examination.

In later stages of this study the selection of isolates was aided by use of the deoxycholate–citrate–mannitol agar medium (Y–M) developed for *Yersinia enterocolitica* isolation by Saari and Jansen (1979). (The potential value of this medium was suggested by J. W. Warren, U.S. Fish and Wildlife Service, Genoa, WI.) The TSB broth enrichments of organs were streaked on Y–M plates, and after 48 h incubation at 20°C, red to magenta colonies were selected and grown on TSA for the cytochrome oxidase test.

Conventional biochemical tests were carried out using the test procedures described by Edwards and Ewing (1972).The production of 2,3-butanediol was measured using the Neish test (Neish 1952) to confirm results obtained in the standard Voges–Proskauer (VP) test. Biochemical tests were also carried out using the API 20E microtube system (Analytab Products, Ltd., St. Laurent, Que.), and the seven-digit biochemical profile numbers were tabulated as described in the API Profile Index. Conventional tests and API strip tests were carried out at both 25 and 37°C.

Antiserum Preparation and Serological Testing

Washed whole cells from 24-h growth on TSA were suspended in 0.5% Formalin–saline for 24 h. Efficacy of killing was checked by streaking the antigen preparation on TSA after collection and resuspension in 0.85% saline. Cell suspensions equivalent to McFarland's number 10 nephalometer concentration were used to inject white rabbits intravenously, according to the following schedule: day 1, 0.5 mL; day 4, 1.0 mL; day 8, 2.0 mL; day 12, 3 mL; day 19, 3 mL. Antibody titers were tested on day 15 and the blood was harvested between days 27 and 29.

Antiserum against the somatic "O" antigens of *Y. ruckeri* strain 9–4–5 was prepared by following the same injection schedule with a McFarland number 10 suspension of cells which had been steamed (100°C) for 40 min and then washed twice before resuspension in saline.

Conjugated antisera for fluorescent antibody tests and standard antisera against *Y. ruckeri*, type I (Hagerman strain, #11.4), type II (Big Creek strain, #11.29), and type III (Australian strain, #7.6) were kindly provided by Dr D. P. Anderson and O. W. Dixon, Biologics, U.S. National Fish Health Research Laboratory, Leetown, W.V.

Slide agglutination reactions and the direct fluorescent antibody test (DFAT) were carried out as described in the American Fisheries Society "Blue Book" (McDaniel 1979). Agglutination titers were determined against twofold dilutions

Table 1. *Yersinia ruckeri* reference strains used in this study.

Culture designation	Initial source	Obtained from
FHL 11.4	Hagerman, Idaho (Serovar I)	U.S. Fish Health Research Laboratory, Leetown, WV
9–4–5	Nova Scotia, 1976 Rainbow trout, hatchery (Serovar I)	B. Zwicker, Fisheries and Oceans Canada, Halifax, N.S.
C-75-199	Mission, B.C., 1975 Rainbow trout, hatchery (Serovar I)	T. Evelyn, Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, B.C.
BC 74	Big Creek, OR, 1974 R. Holt isolate from fall chinook salmon (Serovar II)	J. Rohovec, Department of Microbiology, Oregon State University, OR
ATCC 29473	Type strain (Serovar I)	American Type Culture Collection

of sera prepared with 0.025-mL microdiluters and pipets, using a Cooke microtiter system (Cooke, Alexandria, W.V.). Agglutination titers are expressed as the reciprocal of the last dilution showing a macroscopic reaction. Cells used as antigens were washed in saline, and resuspended in 0.85% saline to an optical density of 1.0 at 520 nm. Heated antigens were steamed for 2 h at 100°C before being washed and resuspended.

Absorbed antisera were prepared by three absorbances at 37°C for 2 h, with 12-h incubation at 4°C following the final absorbance. Cells to be used as the absorbing antigens were washed three times in 0.85% saline and resuspended at a concentration 10 times an optical density of 0.5 at 520 nm. For each absorbance step, cells from 500 μL of the antigen preparations were sedimented in an Eppendorf Microfuge centrifuge, and then resuspended in 500 μL of antiserum. After each absorbance treatment, the cells were pelleted by centrifugation and the serum decanted.

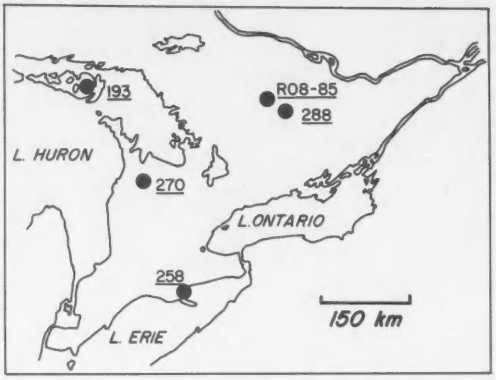

FIG. 1. Southern Ontario showing the distribution of sources of isolates described.

BACTERIAL STRAINS

The *Y. ruckeri* strains included in this study as reference strains are listed in Table 1. We call the Hagerman strain of *Y. ruckeri*, Serovar (Serotype) I, and the O'Leary strain BC74, Serovar II; this is consistent with the code used for antisera and cultures supplied by the Biologics section of the U.S. National Fish Health Research Laboratory, Leetown, W.V.

The Ontario isolate R08-85 included in this study was kindly provided by Carl Lautenschlager, Ontario Ministry of the Environment, Dorset, Ont. This organism was isolated from the lower intestine of an apparently healthy muskrat (*Ondatra zibethica*) trapped near Dorset in December 1978, after a cold-enrichment procedure was used for the detection of *Y. enterocolitica*. Dr W. D. Paterson of Connaught Laboratories, Toronto, provided the cultures of *Vibrio anguillarum* and *Aeromonas salmonicida*. All other bacteria were holdings of the culture collection, Department of Microbiology, University of Guelph (DMG), or new isolates from this study (ONT).

Results

SCREENING AND SELECTION

In initial screening of selected oxidase-negative colonies from TSA plates, we frequently observed slide agglutination reactions with organisms that were later found to be distinct from *Y. ruckeri* in biochemical test results. The most commonly identified cross-reacting organism was *Hafnia alvei*. Strain ONT-203, an isolate from trout, produced an agglutination titer of 160, and *H. alvei* ONT 220, from coho salmon (*Oncorhynchus kisutch*), a titer of 320. The antiserum was used in these screening tests had been prepared against heated cells of *Y. ruckeri* 9−4−5, and gave a titer of 640 against the homologous cells. We subsequently found a fairly high degree of reactivity (titers of 80 or greater) of this anti-"O"-serum with whole cells of *Salmonella arizonae* DMG 602, *Citrobacter freundii* DMG 430, *Enterobacter aerogenes* DMG 622, and *Y. enterocolitica* ONT 207, an environmental isolate. Detectable slide agglutination reactions were also seen with *Aeromonas hydrophila* DMG 352, *V. anguillarum* DI-1, and *A. salmonicida* exATCC 14714. These organisms, while oxidase-positive, could pose problems in fluorescent antibody tests of tissue smears. With the possible exception of *H. alvei* ONT-220, all the above organisms gave negligible titers when tested against antiserum prepared against whole cells of *Y. ruckeri* FHL 11.4.

During a screening period of about 1 yr, four isolates were found that corresponded closely to the biochemical test patterns described for *Y. ruckeri*. Strain ONT-193 was isolated in May 1980, from the kidney of a single yearling hatchery brook trout. Strain ONT-258 was isolated in January 1981 from 3 of 20 rainbow trout (*Salmo gairdneri*) in the brood stock of a hatchery. In two fish, the organism was recovered from the heart only, whereas it was found in the kidney and liver of the third fish. The organism was isolated again 3 wk later in 1 fish out of 12 taken from the same lot. Strain ONT-270 was found in 3 of 30 wild brown trout (*Salmo trutta*) collected in February 1981, in the heart of two fish and the kidney of a third. Strain ONT-288 was one of many other organisms isolated from the liver of one of eight cisco (*Coregonus artedii*) collected in dead or distressed condition in May 1981. These four fish isolates and strain R08-85 came from disperse geographic locations (Fig. 1). There is no evidence to suggest a link to imported fish stocks for any of the isolates.

BIOCHEMICAL CHARACTERIZATION

The biochemical test results for the four fish isolates and the muskrat isolate, R08-85, were similar to the results given by reference strains of *Y. ruckeri* (Table 2). Strains ONT-193, ONT-258, and ONT-288 showed strong positive reactions for sorbitol fermentation, as did strain BC-74 which is representative of the serological variety (Serovar II) described by O'Leary (1977). These strains, and the sorbitol non-fermenting R08-85 strain, all gave positive reactions in the VP and Neish tests within 12−24 h at 25°C. At 37°C all strains in our study gave negative VP and Neish (2,3-butanediol) tests, were nonmotile, and showed delayed

TABLE 2. Biochemical test results for *Y. ruckeri* reference strains and Ontario isolates of 25°C.[a]

Test or substrate	Strain								
	FHL 11.4	C-75-199	9−4−5	BC 74	R08-85	ONT 193	ONT 258	ONT 270	ONT 288
TSI[b]	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A	K/A
H_2S on TSI	−	−	−	−	−	−	−	−	−
Urease	−	−	−	−	−	−	−	−	−
Indole	−	−	−	−	−	−	−	−	−
Methyl red	+	+	+	−	−	−	−	+	−
Voges−Proskauer	−	−	−	+	+	+	+	−	+
Neish test	−	−	−	+	+	+	+	−	+
Citrate (Simmons')	(+)	(+)	+	+	+	+	+	+	+
KCN	+	+	+	+	+	+	+	+	+
Motility (wet mount)	+	+	+	+	+	−	+	−	+
Gelatin liquefaction	−	+	+	+	+	+	+	−	+
Lysine decarboxylase	+	+	+	+	+	+	+	+	+
Arginine dihydrolase	−	−	−	−	−	−	−	−	−
Ornithine decarboxylase	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+
Gas from glucose	−	−	−	−	−	−	−	−	−
Lactose	−	−	−	−	−	−	−	−	−
Sucrose	−	−	−	−	−	−	−	−	−
Mannitol	+	+	+	+	+	+	+	+	+
Sorbitol	w	w	w	+	w	+	+	w	+
Inositol	−	−	−	−	−	−	−	+	−
Arabinose	−	−	−	(w)	(w)	−	−	(w)	−
Maltose	+	+	+	+	+	+	+	+	+
Mannose	+	+	+	+	+	+	+	+	+
Citrate (Christensen's)	+	+	+	+	+	+	+	+	+
Nitrate	+	+	+	+	+	+	+	(+)	+
DNase	−	−	−	−	−	−	−	−	−
Cytochrome oxidase	−	−	−	−	−	−	−	−	−
Malonate	−	−	−	−	−	−	−	−	−

[a]Positive result in 24−48 h, +; weak reaction, w; delayed reactions of 3 d or more, (+) or (w).
[b]K/A indicates an alkaline slant and acid butt on triple sugar iron agar (TSI).

reactions on Simmon's citrate and KCN broth (2−4 d). All other test results at 37°C were essentially the same as at 25°C (data not shown).

We found some difficulty in interpreting the sorbitol fermentation test results for strains R08-85, ONT-270, and reference strains of Serovar I. In fermentation broth base these cultures gave an indicator color reaction intermediate between the acid reaction shown with fermented sugars such as mannitol and glucose, and the neutral reaction given with non-fermented sugars such as lactose and sucrose. We repeated the sugar tests, using semisolid O/F test medium base (Difco), with filter-sterilized sugars added to a final concentration of 1%. In this medium the reference strains of Serovar I showed a distinct acid reaction, which was distinguished from the sorbitol-positive response of BC 74 only by the appearance of a green-blue color at the top of the tube after 48 h or longer. We retested the strains using sorbitol from three different commercial sources, but found no evidence to suggest significant contamination with a readily fermented sugar. The test results for sorbitol fermentation would likely be scored as positive, if it were not for the expectation of a negative reaction from Serovar I strains. With a final concentration of 0.2% sorbitol, positive and negative tests were clearly distinct.

Biochemical test results obtained using the API 20E® system at 25°C clearly identified the reference strains FHL 11.4, ATCC 29473, 9−4−5, and C-75-199, and isolate R08-85 as *Y. ruckeri*, but strain BC 74 and two of the Ontario isolates were identified as species of *Shigella*, and the other two isolates were unidentified (Table 3). Weak reactions for the lysine decarboxylase and Voges−Proskauer (V−P) tests after 24 h at 25°C presented problems in scoring the results for all strains.

SEROLOGICAL REACTIONS

Consistent with biochemical test results, strain R08-85 showed an agglutination titer only with antiserum prepared against strain FHL 11.4 (Serovar I). Strains ONT-193, ONT-258, and ONT-288 differed from previously described strains of *Y. ruckeri* in that they reacted with both type I and type II antisera.

All four fish isolates had initially been selected because they demonstrated slide agglutination with antiserum prepared against whole cell FHL 11.4 antigens. Strain ONT-270, however, appeared to lose its reactivity with type I antiserum after culture, although its biochemical test results had remained consistent, and antiserum prepared against ONT-270 did not significantly cross-react with cells of any other strain (Table 4). While the API 20E biochemical profile identified ONT-270 as *Shigella sonnei* (Table 3), no slide agglutination reactions were observed when ONT-270 cells

TABLE 3. API 20E Biochemical Profile Codes[a] for *Yersinia ruckeri* reference strains and Ontario isolates.

Strain	Profile Code at 25°C	Anomalous results	Identification from API
ATCC 29473	1104100	—	*Y. ruckeri*
FHL 11.4	1104100	—	" "
C-75-199	1104100	—	" "
9−4−5	1104100	—	" "
BC 74	1104500	Sorbitol +	*Shigella sonnei*
R08-85	1104100	—	*Y. ruckeri*
ONT 193	0104500	ONPG −, sorbitol +	*Shigella boydii*
ONT 258	1105500	Sorbitol +, V−P +	(Not listed)
ONT 270	1104102	Arabinose +	*Shigella sonnei*
ONT 288	1105500	Sorbitol +, V−P +	(Not listed)

[a]Profile Codes were obtained as described in the API 20E Analytical Profile Index. Each of the seven-digits in the code number uniquely describes the positive results in a set of three tests, being the sum of the values 1, 2, and 4, assigned to positive reactions in the first, second, and third test, respectively, of each set. The sequence of the tests on the API 20E strip is ONPG; arginine dihydrolase, lysine decarboxylase; ornithine decarboxylase, citrate, H_2S; urea, tryptophan deaminase, indole; Voges−Proskauer, gelatin, glucose; mannitol, inositol, sorbitol; rhamnose, sucrose, melibiose; amygdalin, arabinose, cytochrome *c* oxidase.

were tested with the standard antisera of the Difco-Bacto-*Shigella* antisera set.

Strain R08-85 showed a strong reaction to staining with type I fluorescein-labeled antiserum, while strains ONT-270 and ONT-288 showed some clumps of dark cells in areas of surface brightness. No definitive reactions were seen between type II serum and the new strains, but there was a dim but distinct reaction of ONT-258 with labeled type III (Australian) antiserum. Reference strains FHL 11.4 and BC-74 reacted as expected in control tests.

TABLE 4. Agglutination titers of reference strains and isolates against anti-whole-cell-sera.

Antigen	Titer with antiserum prepared against: FHL 11.4	BC 74	ONT 270
FHL 11.4	640	—[a]	—
ATCC 29473	640	—	—
C-75-199	640	—	—
9−4−5	1280	—	—
BC-74	—	2560	—
R08-85	320	—	—
ONT 193	160	320	80
ONT 258	5120	1280	—
ONT 270	—	—	1280
ONT 288	5120	2560	—

[a]A titer of 20 or less is designated —.

ABSORBED ANTISERA TESTS

Antisera prepared against strains FHL 11.4 and BC-74 were cross-absorbed against each of the Ontario isolates to characterize their serological interactions more precisely. Agglutinating titers of the various strains against these sera are shown in Table 5. Strain R08-85 appears, again, to be properly placed in serovar I, while strains ONT-193, ONT-258, and ONT-288 most closely resembled, but were not identical with, strain BC-74.

The unexpectedly high titers of ONT-258 and ONT-288 cells against anti-FHL 11.4 serum were not observed when heat-treated cells of ONT-258 and ONT-288 were tested

TABLE 5. Agglutinating titers against absorbed anti-*Y. ruckeri*-sera.

Antiserum against:	Absorbing antigen	Titer with various antigens[a] FHL 11.4	BC 74	R08-85	ONT 193	ONT 258	ONT 288	
FHL 11.4 (whole cells)	None	640	—	320	160	20 480	10 240	(10 240)[b]
	FHL 11.4	—	nt	—	—	20 488	10 240	(10 240)
	BC 74	640	nt	320	160	320	160	(160)
	R08-85	—	nt	—	40	10 240	—	(—)
	ONT 193	320	nt	320	—	5 120	—	(10 240)
	ONT 258	640	nt	320	160	—	80	(160)
	ONT 270	640	nt	320	40	10 240	40	(10 240)
	ONT 288	640	nt	320	40	5 120	—	(5 120)
BC 74 (whole cells)	none	—	2560	—	640	2 560	2 560	
	FHL 11.4	nt	2560	nt	640	2 560	1 280	
	BC 74	nt	—	nt	—	80	—	
	R08-85	nt	1280	nt	320	160	320	
	ONT 193	nt	1280	nt	—	640	80	
	ONT 258	nt	1280	nt	320	—	320	
	ONT 270	nt	2560	nt	640	2 560	2 560	
	ONT 288	nt	160	nt	—	640	—	

[a]A titer of 20 or less is designated —; nt = not tested.

[b]Cells of ONT-258 and ONT-288 gave unexpectedly high titers with anti-FHL-11.4 serum. In particular, a light haze surrounding pellets of ONT-288 cells persisted even after serum absorbance, which could be scored as artificially high titers (figures in parentheses).

against the serum, or when the whole cell antigens were tested against anti-"O"-9−4−5-serum (Table 6).

Discussion

The five Ontario isolates described in this study appear to have sufficient similarities to reference cultures of *Y. ruckeri* to suggest that they can be considered strains of this species. Variations in the biochemical test results (Table 3) are with respect to the methyl-red and V−P reactions, motility at 25°C, gelatin liquefaction, and fermentation of sorbitol and arabinose. For each of these tests, examples of both positive and negative test results have been previously reported for different authentic strains (Ross et al. 1966; Wobeser 1973; Ewing et al. 1978), and so the variations observed here appear to fall within the limits of the species description. The negative methyl-red test results and positive V−P reactions shown at 25°C by four of the five isolates were unusual, particularly in the case of strain R08-85, which otherwise corresponded biochemically and serologically with previously described Serovar I isolates (Bullock et al. 1978; Ewing et al. 1978). A single strain described in the study by Ewing et al. (1978) was V−P-positive.

Sorbitol fermentation has been suggested as being a characteristic of the second serological variety of *Y. ruckeri*, represented by reference strain BC 74 (O'Leary 1977). False-positive sorbitol reactions, such as described here, could present a major diagnostic problem, as present evidence suggests that the sorbitol-positive strains of Serovar II are not associated with disease and are less virulent than strains of Serovar I, the Hagerman variety (G. L. Bullock, E. B. Shotts and C. Starliper, Joint Meeting of the Fifth Annual Fish Health Section/AFS and the Sixth Annual Eastern Fish Health Workshop, Mississippi State, MS, 1981, p. 53−54). The appearance of a weak sorbitol fermentation reaction could be the result of prolonged culture on laboratory media, as has been observed to occur with some pathogenic strains of *Escherichia coli* (Rappaport and Henig 1952). Alternately, the close similarity of the chemical structure of sorbitol to that of mannitol, which is fermented by all strains of *Y. ruckeri*, could allow illicit transport and fermentation of sorbitol by the mannitol-specific enzymes, particularly at high substrate levels. The significance of the test result in the diagnostic process provides reason for concern that the procedure used be uniform and that reference strains be used to interpret the reactions obtained.

The API 20E biochemical test strips provide a convenient tool for examining isolates in fish diagnostic laboratories, but the API 20E Analytical Profile Index® data base appears not to include strains with anomalous V−P and sorbitol reactions, and so certain strains, such as BC 74, are not identified as *Y. ruckeri*. The negative citrate and lysine decarboxylase API® reactions after 24 h at 25°C are at significant variance from the conventional test results obtained in this study and by others (Ewing et al. 1978). The combinations of results from the conventional tests for lysine and ornithine decarboxylase, Simmon's citrate, gelatin liquefaction, motility, and sorbitol and arabinose fermentation suggest that strain BC 74 and the four Ontario fish isolates are more similar to *Y. ruckeri* than to the species of *Shigella* suggested by the API® biochemical profiles. Caution in interpretation of results appears warranted when rapid biochemical test systems, largely designed for use at 37°C, are used to identify fish pathogens.

TABLE 6. Agglutinating titers of whole cell and somatic antigens with anti-*Y. ruckeri*-sera.

Antigen		Titer with antiserum prepared against: FHL 11.4	BC 74	9−4−5-"O"
FHL 11.4	Whole cells	10 240	—	1280
	Heated	640	—	640
BC 74	Whole cells	—	10 240	—
	Heated	—	640	—
ONT 258	Whole cells	20 480	10 240	—
	Heated	—	320	80
ONT 288	Whole cells	5 120	5 120	320
	Heated	40	640	40

Because serological reactions are extensively used in diagnosis and detection of the enteric redmouth bacterium, the type of antiserum to be used should be specifically stated in procedure descriptions. The cross reactions between anti-"O"−*Y. ruckeri*-serum and *H. alvei* reported here have also been observed by other laboratories (American Fisheries Society, Fish Health Section Newsletter, 1978, Vol. 6, No. 1). We have attempted without success to isolate *Y. ruckeri*-specific bacteriophage as an additional diagnostic aid, although phage specific for some strains of *H. alvei* were found (R. Stevenson, R. Bennett, and S. French, Department of Microbiology, University of Guelph, Guelph, Ont. N1G 2W1, unpublished data).

Agglutinating titers with specific antisera (Table 4) and the results of tests with absorbed sera (Table 5) confirmed the identity of strain R08-85, the muskrat isolate, as *Y. ruckeri* Serovar I. Three of the fish isolates, ONT-193, ONT-258, and ONT-288, were unusual in that they reacted to varying degrees with both type I and type II antisera (Table 4), whereas cross-absorbance tests suggested that these strains possess antigenic determinants additional to those previously described (Bullock 1978; O'Leary 1977). The DFAT strains of the four Ontario fish isolates were difficult to interpret as cells did not appear to react as strongly as expected from agglutinating titers. On the basis of the DFAT reaction, strain ONT-258 may have some serological similarities to the third, Australian variety of *Y. ruckeri*, described by Bullock et al. (1978). However, more detailed examination of the antigenic components of all the strains appears to be required before relationships among the serovars can be established. It is possible that surface or capsular material may be involved in some of the DFAT and agglutination reaction observations. The significant drop in agglutination titers shown by ONT-258 and ONT-288 after heat-treatment (Table 6) and the relatively low reactivity between whole cells of these strains and anti-"O"-9−4−5-serum (Table 6) would be consistent with the presence of a heat-denatured surface antigen.

The *Y. ruckeri* isolates described here suggest that there is greater diversity in the serological and biochemical properties of strains of this species than previously believed. This has some significance for diagnosis, but the result from this study

with greatest implication for fish health protection is that bacteria identifiable as *Y. ruckeri* can be isolated from disperse locations in a large geographic area where there has been no evidence of enteric redmouth disease. At present, fish health protection regulations do not distinguish between varieties of *Y. ruckeri*, and more information regarding the relative roles of bacterial, host, and environmental factors in enteric redmouth disease is needed to assess the significance to be attached to isolations of strains other than the Hagerman variety (Serovar I).

Acknowledgments

We are very grateful for the financial support of the Canadian National Sportsmen's Fund for this study. Additional financial support and other assistance were provided by the Ontario Ministry of Natural Resources. The help and advice of Dr R. J. Douglas, Prof R. A. Johnston, Ms Brenda Allan, and Ms Sharon French was appreciated.

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Alterations in the Cellular and Subcellular Structure of Marine Teleosts and Invertebrates Exposed to Petroleum in the Laboratory and Field: A Critical Review

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Laboratory studies with individual petroleum components, model mixtures of hydrocarbons, fractions of petroleum, and whole oil have demonstrated, mostly at high exposure concentrations, an association between petroleum and various cellular/subcellular alterations in marine organisms. Recent evidence, notably from studying the effect of the AMOCO CADIZ oil spill, suggest that cellular/subcellular alterations also result from exposing marine organisms to petroleum in the field. In marine environments, however, it is still generally not possible to predict the type or degree of impact of petroleum on organisms because little information is available on the influence of myriad natural and man-induced environmental variables on its fate and effects.

Key words: marine invertebrates and teleosts, petroleum, pollution, aromatic hydrocarbons, acute/chronic exposures, cellular/subcellular changes, pathological changes, morphology, mutagenesis, DNA binding, laboratory studies, field studies

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Des études en laboratoire avec composantes individuelles du pétrole, des mélanges modélisés d'hydrocarbures, des fractions de pétrole et du pétrole entier ont démontré, surtout à de fortes concentrations d'exposition, une association entre le pétrole et diverses altérations cellulaires/subcellulaires chez des organismes marins. Selon de récentes données, recueillies principalement au cours d'études sur les effets du déversement accidentel de l'AMOCO CADIZ, les altérations cellulaires/subcellulaires résulteraient également de l'exposition d'organismes marins à du pétrole sur le terrain. Il est toutefois encore impossible de prédire, dans les environnements marins, le type ou le degré d'impact dupétrole sur les organismes. Nous ignorons encore à peu près tout de l'influence des innombrables variables de l'environnement, naturelles ou causées par l'homme, sur le sort du pétrole et de ses effets.

Received August 27, 1981
Accepted February 12, 1982

Reçu le 27 août 1981
Accepté le 12 février 1982

EACH year an estimated four million metric tons of petroleum enter the marine environment through sea and land-based discharges (e.g. tanker spills), atmospheric fall-out, and other events (Clark and MacLeod 1977). These occurrences lead to a concern about petroleum-related alterations in marine life, such as changes reflected in cellular/subcellular structure. Because of the obvious importance of such alterations, an assessment is made of laboratory and field studies relating to gross pathological, histopathological, and selected correlated molecular perturbations that result from exposing marine organisms to petroleum.

Various types of organisms have been exposed. Usually, the conditions used in each study differed substantially, and relationships between petroleum or its components and biological changes were often poorly defined. Nevertheless, important information has been obtained on the nature of the cellular/subcellular changes occurring in organisms exposed in the laboratory and field, although little is known about how the morphological changes actually affect the health and survival of organisms. Overall, serious questions can be raised about the relevance of the conditions (e.g. exposure concentrations) used in laboratory studies in relation to understanding the effects of petroleum in the field. In this regard, excluding cataclysmic releases of petroleum in confined areas, concentrations of total hydrocarbons (determined by gas chromatography) seldom exceed 1 mg/L in oil-polluted waters; in contrast, concentrations of total hydrocarbons in marine sediments have been shown to exceed several thousand milligrams per kilogram (Clark and MacLeod 1977).

Printed in Canada (J6615)
Imprimé au Canada (J6615)

TABLE 1. The effects of aromatic hydrocarbons on lysosomal stability in the digestive cells of mussels *Mytilus edulis*. (From Moore 1979.)

Treatment (100 µL injected per mussel)	Labilization period of latent lysosomal hexosaminidase as % of the vehicle control[a]	Probability[b] (U-test)
Vehicle Control I (liquid paraffin)	100.0±5.3	
Vehicle Control II (liquid paraffin)	97.8±5.4	>0.05
Naphthalene (10^{-2} mol/L)	108.7±4.4	>0.05
2-Methylnaphthalene (10^{-2} mol/L)	73.9±8.7	<0.05
2,3-Dimethylnaphthalene (10^{-2} mol/L) I	43.5±4.4	<0.01
2,3-Dimethylnaphthalene (10^{-2} mol/L) II	52.2±5.2	<0.01
Phenanthrene (10^{-2} mol/L)	69.6±4.3	<0.01

[a]Mean ±SE, five animals per sample.
[b]Comparing treatments with Vehicle Control I (liquid paraffin).

In the present paper, an evaluation of the relevent data base is presented, together with a perspective of its strengths and limitations.

Individual Petroleum Components and Model Mixtures of Hydrocarbons: Laboratory Studies

Morphological alterations associated with exposing marine organisms to individual hydrocarbons and to model mixtures have been identified in laboratory studies. Information from these investigations is useful in pinpointing biological structures affected and, to a limited extent, in differentiating these impacts from those caused by other environmental chemicals. A variety of organisms, including early developmental stages that are particularly sensitive to petroleum exposure, were used in these types of exposure studies.

Mummichogs (*Fundulus heteroclitus*) were exposed to naphthalene in salt water at concentrations ranging from 0.002 to 30 mg/L, the latter value substantially exceeding concentrations generally found in marine environments (DiMichele and Taylor 1978). Alterations, notably ischemia, in brain, liver, and pancreas of these fish occurred after 15 d with exposures to concentrations of 0.2 mg/L and higher. There was damage to neurosensory organs (e.g. lateral line necrosis) at exposures as low as 0.02 mg/L for the same period. Also, the lower concentrations resulted in elevations in serum glucose, protein, and cortisol.

Young chinook salmon (*Oncorhynchus tshawytscha*) received a model mixture of aromatic hydrocarbons (2-methylnaphthalene, 2,6-dimethylnaphthalene, 2,3,6-trimethylnaphthalene, fluorene, phenanthrene, 1-phenyldodecane, and heptadecylcyclohexane) and 2,3-benzothiophene in the food at 5 mg/kg daily for 28 d (Hawkes et al. 1980). The intestinal mucosa exhibited little exfoliation of cells or necrosis, but varying degrees of subcellular damage occurred in eight of nine fish examined. These aberrations were principally unusual inclusions in the columnar cells of the mucosa, and vesiculation of cytoplasm near the luminal surface of columnar cells. Animals with the greatest amount of vesiculation showed basal mucosal cells with an unusual electron-transparent cytoplasm and an increase in the granular endoplasmic reticulum.

Pacific herring (*Clupea harengus pallasi*) embryos and larvae were exposed to ~0.9 µg/L of benzene in seawater for 24 h (Eldridge et al. 1977). This exposure resulted in delayed overall embryonic development.

Phenol, an oxidation product of benzene in marine environments, induced cytological damage (disintegration of cytoplasmic components of gill, digestive gland, and hindgut) in adult clams (*Mercenaria mercenaria*) exposed through seawater at 0.1 mg/L and above (Fries and Tripp 1977). In addition, at higher concentrations (e.g. 10 mg/L), cell membranes in the tissues ruptured and sloughing of epithelial cells occurred in each organ examined. Whether or not phenol treatment and the resultant loss of epithelial cells results in a greater susceptibility to infection remains unanswered.

Studies were conducted with mussels (*Mytilus edulis*) on the effect of anthracene on the cytology and cytochemistry of pericardial gland cells and their lysosomal responses. Injection of anthracene at the high dose level of 100 µg/mussel (50–60 mm in length) brought about an increase in free hexosaminidase in lysosomes of the pericardial gland cells, indicating that anthracene induces a destabilizing effect on these structures (Moore et al. 1980). Previous work demonstrated the importance of the lysosomal–vacuolar system in stress responses in the kidney and digestive cells of *M. edulis*. For example, anthracene (10–100 µg/mussel; 50–60 mm in length) induced dose-dependent lysosomal destabilization and release of hexosaminidase in the digestive cells of *M. edulis* after 24 h. Destabilization was associated with cytolysis. The destabilizing effect of 100 g of injected anthracene lasted for 96 h; however, a reversion to the condition of controls occurred by 168 h. The study raised the possibility that aromatic hydrocarbons interfere with hormonal action because similar cytological effects are associated with estradiol-17 β administered to bivalves (Moore et al. 1978a, b).

Phenanthrene and methylated naphthalenes also caused lysosomal destabilization in *M. edulis*, which apparently was directly related to mechanisms of cytotoxicity in digestive cells; 2-methylnaphthalene, 2,3-dimethylnaphthalene, and phenanthrene injected at 0.1 mL of a 10^{-2} mol/L solution per mussel all induced statistically significant decreases in lysoso-

mal stability (Table 1) (Moore 1979).

Sea urchin (*Paracentrotus lividus*) eggs exposed to 5 μg/L (fertilized) and 4 μg/L (unfertilized) of benzo[a]pyrene for 3 h exhibited abnormal cleavage, and the embryos developed into irregular morulae (Ceas 1974). This abnormality may be associated with an interaction of the activated hydocarbon or its metabolites with DNA which resulted in altered replication.

The question of whether marine organisms convert aromatic hydrocarbons to metabolites that bind with DNA has been addressed in considerable detail (Varanasi and Gmur 1980; Varanasi et al. 1980). Metabolic activation and subsequent binding of benzo[a]pyrene metabolites to deproteinized salmon sperm DNA were investigated in the presence of liver homogenate fractions (10 000 × *g* supernatant) from untreated, 3-methylcholanthrene- or benzo[a]pyrene-treated starry flounder (*Platichthys stellatus*). English sole (*Parophrys vetulus*), and coho salmon (*Oncorhynchus kisutch*). It was demonstrated that benzo[a]pyrene was converted in vitro to certain reactive metabolites that bind to DNA. Additional studies on the metabolic activation of aromatic hydrocarbons and binding of metabolites to DNA showed that both processes occur in vivo in fish exposed to benzo[a]pyrene in the laboratory (Table 2) (Varanasi and Gmur 1981a, b; Varanasi et al. 1981), and that binding is also enhanced by prior exposure to Prudhoe Bay crude oil and PCBs (Varanasi et al. 1980; Egaas and Varanasi 1982).

Laboratory studies indicate, therefore, that individual hydrocarbons and model mixtures of hydrocarbons, and possibly certain oxidation products of hydrocarbons, are associated with a number of cellular and subcellular alterations in marine organisms, especially in early developmental stages; however, the exact exposure conditions that lead to these anomalies are not clearly understood. Moreover, studies of the interactions of individual petroleum hydrocarbons and/or metabolites with nucleic acids suggest that alterations in the biochemistry and structure of DNA/RNA occur; however the implications of the findings to organism viability in either the laboratory or field are unknown.

Petroleum and Petroleum Fractions: Laboratory Studies

A number of investigations have addressed the question of whether complex mixtures of petroleum components bring about alterations in the tissues of marine organisms at the cellular and subcellular level. These studies, again often employing incredibly high-exposure concentrations, have included selected vertebrates and invertebrates in their adult and early developmental stages.

Alterations in Cellular and Subcellular Structure

English sole, starry flounder, and rock sole (*Lepidopsetta bilineata*) were exposed to sediment contaminated with Prudhoe Bay crude oil (0.2, 0.5, and 1.0%, v/v) for 3 wk to 4 mo (McCain et al. 1978; McCain and Malins 1982). The rock sole and starry flounder exhibited no apparent petroleum-associated effects; however, histological, hematological, and physiological changes were observed in English sole. Exposure for 4 mo to a sandy sediment contaminated with 0.2% Prudhoe Bay crude oil resulted in the following petroleum-related changes in English sole: (1) during the 1st month, when sediment and tissue concentrations of aromatic hydrocarbons were highest, 50% of the exposed fish and none of the controls had severe hepatocellular lipid vacuolization, with greater than about two thirds of the hepatocyte cytoplasm occupied by lipid vacuoles; (2) the number of fish regaining all or part of an initial loss of weight was statistically significantly lower in the experimental (16 fish) than in the control (15 fish) groups after 4 mo of exposure; and (3) although no control fishes died (except for those purposely killed) and all looked normal during the 4-mo experiment, 18% (3 of 17 fish) of the oil-exposed fish died, or were moribund and appeared extremely emaciated. Other experiments of shorter duration (3−12 wk) conducted on English sole exposed to 0.5 and 1.0% Prudhoe Bay crude oil under comparable conditions resulted in significant alterations in hemoglobin and hematocrit values; however, mortalities and weight losses were not observed (McCain and Malins 1982).

TABLE 2. Distribution of hydrocarbon-derived radioactivity in liver and bile of English sole exposed to benzo[a]pyrene (BaP) in sediment.[a] (From Varanasi and Gmur 1981b.)

Hydrocarbon and metabolites	Liver[b]	Bile[b]
Unmetabolized B[a]P	2	2±3[c]
Ethyl acetate soluble metabolites	16	14±10
Glucuronides	<5	43±3
Sulfates	<5	13±3
Other water-soluble metabolites	45	28±2
Bound to cellular constituents	34	—

[a]Fish were exposed to ^{3}H-B[a]P (1.8 nmol/g) in sediment containing 1% Prudhoe Bay crude oil.

[b]Values, expressed as percentage of total radioactivity, are from a pool of three samples.

[c]Each entry is the mean ±SD of three values.

Gross morphological changes occurred in cunner (*Tautogolabrus adspersus*) exposed for 2 wk to seawater containing a surface slick of Venezuelan crude oil (Kiceniuk et al. 1980). Exposure concentrations were not defined; however, decreases in spleen weights in female fish were found together with increases in gallbladder weights in both sexes. Another study with cunner exposed to Venezuelan crude oil (exposure concentrations also not determined) for 6 mo revealed statistically significant differences in testes/somatic indices, and in eye lens diameters which may have resulted from hydration and may represent a reversible condition (Payne et al. 1978a). No histopathological changes in spleen, kidney, heart, gonads, gill, or muscle were observed (the gallbladder was apparently not examined).

Dramatic increases in the rough endoplasmic reticulum of liver cells of rainbow trout (*Salmo gairdneri*) were observed after exposure to higher doses (1 g/kg food) of Prudhoe Bay crude oil administered in the diet for 8 mo (Hawkes 1977). This exposure also resulted in eye lens abnormalities (alterations in lens diameters) comparable to those found in mammals exposed to naphthalene. In this regard, it is interesting

TABLE 3. Summary of gill morphology in rainbow trout (*Salmo gairdneri*) following 7 d of oil treatment, comparing different modes of exposure. (From Engelhardt et al. 1981.)

Exposure mode		Degree of fusing of lamellae	Epithelial separation	Cl-cell[a] exposure	Cl-cell location	Cl-cell vacuoles
Fresh water	Control	None	None	None	Basal	Rare to none
Emulsion	Paraffin	None	None	Rare	Basal	None
	NW[b]	Some	Some	Extensive	Length[f]	Extensive
	VEN[c]	Extensive	Extensive	Extensive	Length	Some
WSF[d]	NW	None	None	Rare	Basal	Few to none
	VEN	None	"	Little	Basal	None
i.p.[e]	NW	Little	"	Some	(Basal)[g]	Rare
Sea water	Control	Rare	"	None	Basal	None
Emulsion	NW	Some	"	Little	(Basal)	Some
	VEN	Variable	"	Some	(Basal)	Common
i.p.	NW	Rare	"	None	Basal	None

[a]Chloride-type cell; increase in chloride cell apical exposure through epithelial layer.
[b]Norman Wells crude oil, weathered 3 d to 200 μL/L.
[c]Venezuelan crude oil, weathered 3 d to 200 μL/L.
[d]Water-soluble fraction.
[e]Intraperitoneal injection, 100 μL/kg fish weight per day.
[f]Cl-cells found along length of secondary lamellae.
[g]Cl-cells found in basal region and extending part way up secondary lamellae.

to note that Van Heyningen (1970) showed that rabbits fed naphthalene readily develop cataracts.

Marked gill damage was observed in immature rainbow trout exposed to a freshwater emulsion comprising Venezuelan crude oil weathered for 3 d (200 μL/L) (Engelhardt et al. 1981). The effects consisted of epithelial separation, chloride cell abnormalities, and fusion of secondary lamellae. Notably, comparable exposures in salt water resulted in little epithelial detachment (Table 3). Hydromineral imbalances (e.g. Na^+, K^+, and Ca^{2+}) were believed to be primarily related to the gill lesions, although cortisol imbalances and possible ATPase inhibition may have been influencial. In another study with rainbow trout, weathered Norman Wells and Venezuelan crude oils (100 μL/L; in the suspended particulate form) brought about chloride cell modifications, epithelial separation, and curling of the primary lamellae of the gills (Wong et al. unpublished data). These changes were similarly believed to be associated with observed ionic imbalances.

The liver of winter flounder (*Pseudopleuronectes americanus*) exposed to 12 mg/L of No. 2 fuel oil in seawater for 3 d to 8 wk, and to 90 mg/L for 4-11 wk, showed no histopathological changes; however, the growth and survival of larvae from exposed fish were decreased in comparison to the control group (Kuhnhold et al. 1978). Morphological changes were observed in the gills of rainbow trout exposed to the inordinately high concentration of 200 mg/L of seawater-accommodated Bunker C oil for 96 h. The gill damage included curling of the lamellae tips, necrosis, and rupture of cells (McKeown and March 1978).

Waste motor oil (20 mg/L and higher!) in seawater induced dark pigmentation bodies in the vascular systems of Atlantic silversides (*Menidia menidia*), oysters (*Crassostrea virginica*), and scallops (*Aquipectins irradians*) when exposed for various periods (e.g. 60 d) (Gardner et al. 1975). These alterations, which may not be detrimental, were found in the pseudobranch, heart, and arterial system of the Atlantic silversides and the branchial vasculature of the oysters. Branchial and renal lesions (necrosis) were found in the scallops.

Atlantic silversides were also exposed for 168 h to Texas–Louisiana crude oil in seawater (~0.14 mg/L) and to saltwater-"insoluble" (~0.58 mg/L) and saltwater-soluble components (~0.167 mg/L) of the oil (Gardner 1975). Crude oil was linked to hyperplasia of the olfactory sustentacular, or supporting epithelium. Increased cellularity resulted in "nodules" extending into the olfactory lumina. Also, large areas of the olfactory mucosa underwent severe degeneration. Necrocytosis encompassed neurosensory and sustentacular epithelium. Damage to the heart occurred, notably to the ventricle (e.g. foci of necrotic myocardium). The saltwater-insoluble fraction produced effects similar to those of the crude oil as well as vasodilation in olfactory submucosa. However, the saltwater-soluble fraction induced olfactory lesions different from those associated with the other two fractions. For example, epithelial metaplasia was observed and there was a replacement of neurosensory, sustentacular and mucous cells by less-differentiated cell types.

Black Sea flounder (*Platichthys flesus*) embryos were exposed to a number of concentrations (up to 2.5 mg/L) of "dissolved petroleum products" from crude oil (unspecified) for up to 72 h (Mazmanidi and Bazhashvili 1974). Each of the concentrations, except the low value of 0.025 mg/L, brought about alterations in the shapes of pigment cells; however, the findings are of limited value because the experimental conditions were unclear and the significance to organism viability of the alteration in the pigment cells is unknown.

The effects of a simulated crude oil spill on the embryonic development of Pacific herring were studied (Smith and Cameron 1979). Of particular interest were the effects of the low-boiling, water-soluble hydrocarbon components of Prudhoe Bay crude oil on embryos. Initial hydrocarbon concentrations were less than 1 mg/L and exposures from 48–144 h led to a high incidence of gross morphological

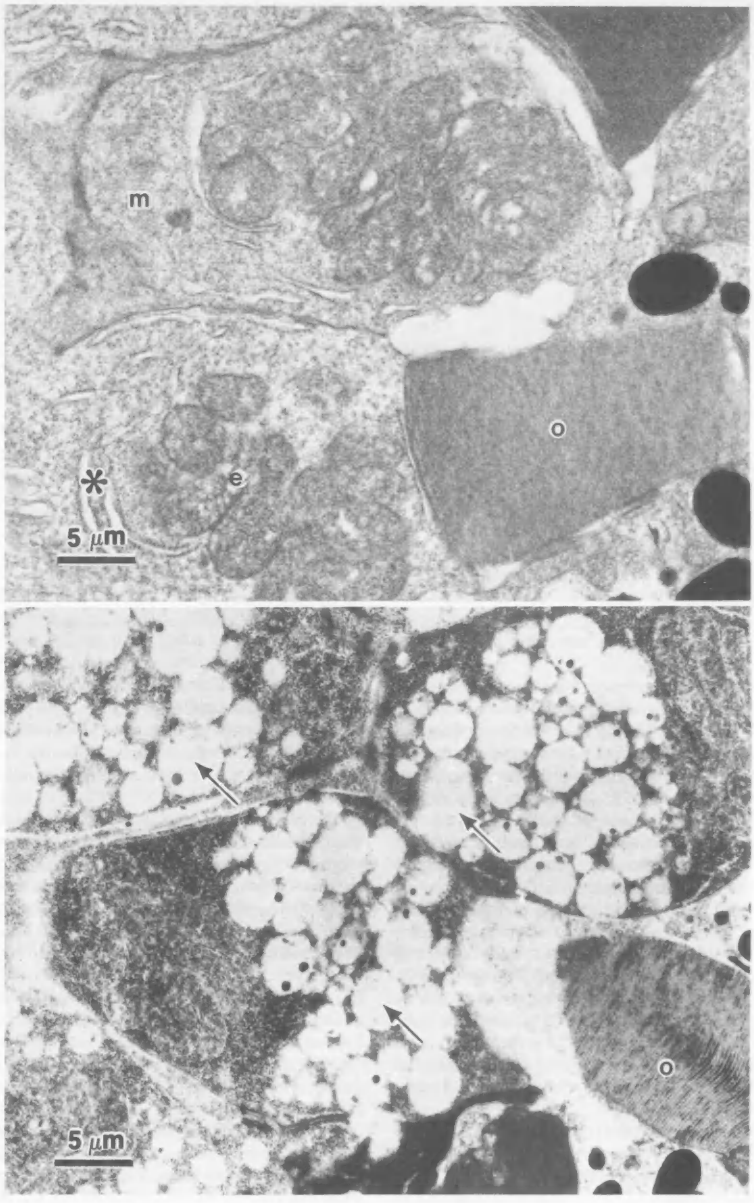

FIG. 1. *Upper* portion: enlargement of receptor cells in a coastal surf smelt (*Hypomesus pretiosus*) embryo. Note the endoplasmic reticulum (*) of the myoid region (m) of the inner segment. *Lower* portion: Receptor cells of surf smelt embryo exposed to 113 μg/L of Cook Inlet crude oil as a seawater-accommodated fraction. The outer segments (o) show little, if any, damage; however, both the myoid (m) and ellipsoid (e) regions of the inner segments are vacuolated (arrows).

TABLE 4. Morphological abnormalities in oocytes from flathead sole force-fed corn oil only or 4.0 mg BaP plus corn oil and fertilized and reared in benzo[a]pyrene-free seawater. (From Hose et al. 1981.)

Group	Percentages of abnormalities found	Hours after fertilization	No. and type of abnormality
Control	1.6	96	1 with abnormally small yolk sac and vesicles in head; 1 with accessory tail
		120	1 with pectoral fins placed more caudally than normal
BaP-treated	5.6[a]	72	2 with abnormally small yolk sacs; 1 with ventral nodule; 1 with constriction before blastopore, small yolk sac; 1 with bifid tail, small yolk sac
		96	1 with bifid tail
		168	1 with no auditory vesicles, small yolk sac, indistinct lenses, and asymmetric pectoral fins
		240	1 with bent notochord
		264	1 with degenerate fin fold

[a]Chi-square test, $P = 0.051$.

abnormalities compared to controls (Smith and Cameron 1979). The abnormalities included pectoral fin erosion and fins that failed to differentiate from the lateral line body wall. Various abnormalities of the mouth were also observed, including "a misfit of the lower jaw into the upper, missing premaxillary bones and failure of the jaw to fully differentiate." In addition, electron microscopy revealed the absence of branchiostegal membranes. There was also evidence of abnormal neural development such as reduced cephalization. In the same experiment embryos of Pacific herring were also exposed to the water-soluble fraction of Prudhoe Bay crude oil for 4–144 h, then returned to clean seawater for further development (Cameron and Smith 1980). Newly hatched larvae from both control and experimental groups showed no gross abnormalities; however, electron microscopy revealed abnormal inter- and intra-cellular spaces in brain and muscle tissues of exposed organisms, whereas no such lesions appeared in controls. Moreover, many mitochondria in body muscle of exposed organisms were swollen and some contained deteriorated cristae.

Surf smelt (*Hypomesus pretiosus*) embryos were exposed to 0.054 mg/L or 0.113 mg/L of the seawater-accommodated fraction of Cook Inlet crude oil for 3 h/d, beginning 6 d after fertilization (Hawkes and Stehr 1982) (Fig. 1). A number of tissues of 21- and 27-d-old exposed embryos were examined by light and electron microscopy for morphological features that differed from those of the controls. Necrotic neurons were observed in the forebrain and the neuronal layer of the retina in both the 21- and 27-d-old exposed smelt, and these were the only tissues with structural changes. The retinal receptor cells appeared normal in 21-d-old exposed smelt; in contrast, in 27-d-old treated embryos there were marked aberrations in receptor cell inner segments. In particular, the cytoplasm of the inner segment was filled with vacuoles and the mitochondria appeared to be in various stages of lysis. Hatching success in the oil-exposed groups was 40% lower than that of the controls.

The gross morphological effects of the water-soluble fraction of Venezuelan heavy fuel oil (No. 6) on cod (unspecified species) eggs and larvae were studied (Kuhnhold 1978). Larvae were exposed at initial concentrations in seawater of 10, 100, and 500 mg/L. These high concentrations of petroleum resulted in moribund larvae that had an abnormally developed apical part of the primordial fin fold. The fins, in later developmental stages, were swollen and showed a more irregular surface structure than in normal larvae. The frequency and degree of these abnormalities appeared to correlate with the oil concentrations.

Gulf killifish (*Fundulus grandis*) embryos were exposed to 1.1, 2.2, and 4.4 mg/L of the water-soluble fraction of No. 2 fuel oil (Ernst et al. 1977). The embryos of these fish were then examined histologically and compared to unexposed control embryos. Only normal tissues were observed in embryos of controls and those exposed to 1.1 mg/L of the petroleum fraction; however, exposure to 2.2 mg/L and above resulted in pathological lesions of organs such as the liver (e.g. necrosis), kidney (underdeveloped with no lumina), and eye (vacuolated retinal cells). The authors stated that "concentrations of WSH (water-soluble fraction) required in the present investigation to produce histopathological lesions in *Fundulus* embryos were much higher than any likely to be encountered in nature, even under a massive oil spill."

TABLE 5. Mutagenic activities and aromatic hydrocarbon content of various oils.[a] (Adapted from Hermann et al. 1980.)

Oil no.	Origin	Mutagenic[b] activity (rev·μL^{-1})	PAH content (μg·L^{-1})	BaP content (μg·L^{-1})
0	White oil	<1	0.64	<5
1	Unused hardening oil	<0.5	2.6	<5
2	Used hardening oil (derived from No. 1)	250	8.3	25 000
3	Unused crankcase oil	<30	nd[c]	<5
4	Used crankcase oil (derived from No. 3)	550	nd[c]	62 000
5	Petroleum distillate	2800	78	840
6	Solvent-refined oil (derived from No. 5)	340	20	<10
7	Petroleum distillate	1200	54	<10
8	Solvent-refined oil (derived from No. 7)	230	9.7	<10

[a]All values are expressed per unit volume of oil. Corresponding weight values may be calculated by using a mean density value of 0.85.

[b]Average of all the determinations obtained in the method of oil dispersion with Tween 80. Oils were all tested in a dose range from 0.1 nL to 20 μL.

[c]nd, no determination was done because the oils concerned contained additives which might have been extracted with the PAH.

Female flathead sole (*Hippoglossoides elassodon*) were force-fed benzo[a]pyrene in corn oil (average dose = 8 mg/kg of body weight) (Hose et al. 1981). Almost four times as many grossly visible lesions were found in eggs from fish exposed to the benzo[a]pyrene compared to controls. Most of these alterations arose between blastopore closure and hatching. The anomalies were mostly malformations of the tail region or yolk sac, as shown in Table 4. These same types of abnormalities were also seen in developing California grunion (*Leuresthes tenuis*) eggs maintained on benzo[a]pyrene-treated sand (Puffer 1979).

Soft-shell clams (*Mya arenaria*) exposed for 28 d to the high concentration of 100 mg/L or less of No. 2 fuel oil in seawater had no marked damage; however, certain aberrations (generalized leukocytosis) were observed (Stainken 1976). Progressive decreases in the concentrations of hydrocarbons in the water (to less than 1 mg/L after 3 wk) over the course of the experiment clouded any attempt to relate hydrocarbon concentration to morphological change.

Morphological changes were observed in the digestive tubule epithelial cells of *Mytilus edulis* after long-term (34−182 d) exposure to the water-accommodated fraction of North Sea crude oil (30 μg/L total oil-derived aromatic hydrocarbons) (Lowe et al. 1981). The changes included a reduction in the height of the digestive cells beyond that shown in a normal feeding cycle. There were also losses in the normal synchrony of the digestive cells and radical alterations in the structures of secondary lysosomes within digestive cells.

Exposure of *M. edulis* larvae to Arabian light oil (2 mL/L in seawater!) resulted in morphological damage (Le Pennec and Le Roux 1979). Larval development was perturbed so that most contaminated larvae showed substantial aberrations in hinge structure and crystallization of the larval or prodissoconch shell. The high exposure concentration used in the work detracts from the significance of these findings.

The effects of Cook Inlet crude oil (containing the high exposure concentration of 133 mg/L of naphthalene equivalents) in seawater on gill, antenna, and hepatopancreas of king crabs (*Paralithodes camtschatica*) were studied (Smith 1976). Gill epithelial cells in animals exposed for 3 d showed electron-dense nuclei, vacuolization, and morphological changes in the endoplasmic reticulum; a predominance of electron-dense cells and increased vacuolization characterized the antennae. After 3 h the hepatopancreas showed increases in lipid vacuoles and the formation of electron-lucent areas in cells that line the lumen. After 80 h of exposure, followed by 64 h in clean water, the morphological alterations associated with lipid appeared to be accentuated. Moreover, basal cells of the hepatopancreas showed an increase in large vacuoles and rounding of the smooth endoplasmic reticulum cisternae.

Petroleum (API reference South Louisiana crude) concentrations of 0.1 and 1.0 mg/L, as strongly agitated seawater emulsions, brought about alterations in pigment cells of American lobsters (*Homarus americanus*) exposed for up to 15 d (Forns 1977). Chromatophores, which are normally contracted in control organisms, were considerably distended in oil-exposed larvae, especially those exposed to 1.0 mg/L. An obvious reddening effect was attributed to diffusion of pigment through the chromatophores.

Studies on the effects of remarkably high concentrations of Kuwait and Ekofisk crude oils (1000−20 000 mg/kg of seawater-accommodated petroleum) on sea urchin (*Psammechinus miliaris* and *Paracentratus lividus*) gametes and embryos did not reveal any alterations in the cytoplasm; however, a number of effects on differentiation were noted (e.g. plutei that were rudimentary), particularly of the skeleton (Lonning and Hagstrom 1975). Comparable results were obtained in other studies of morphological changes associated with exposing sea urchin eggs to petroleum components (Falk-Peterson 1979; Lonning 1977). Embryos of the sea urchins, *Strongylocentrotus droebachiensis* and *S. pallidus*, were exposed to Ekofisk crude oil and various commercial oil

products as water-accommodated fractions (1−47 mg/L based on hydrocarbon content) (Falk-Peterson 1979). No differences between controls and treated larvae were found in surface structure and ciliation; however, at the higher concentrations increased aggregation of electron-dense granules in cells of the outer wall and intestines were noted. Ekofisk oil exposure also resulted in abnormalities at the late gastrula stage, particularly at the prisma and early pluteus stages. The larvae gradually filled with degenerating cells and differentiation of the intestine and skeleton was inhibited.

Rinkevich and Loya (1979) exposed hermatypic coral (*Stylophora pistillata*) to Iranian crude oil (3 mL/L for 2−6 mo). They found that these conditions resulted in a statistically significant decline in the number of female gonads per polyp. Other findings (Rinkevich and Loya 1977) suggest that apparently deleterious biological changes are associated with the exposure of coral to petroleum, such as a decrease in colonization.

Caribbean coral (*Manicina areolata*) exposed to the water-accommodated fraction of No. 2 fuel oil (0.15 ± 0.10 mg/L or less) for 12 wk revealed impaired development of reproductive tissues, degeneration, and loss of symbiotic zooxanthellae and atrophy of mucous secretory cells and muscle bundles (Peters et al. 1981). However, problems in maintaining constant experimental conditions (e.g. concentrations of hydrocarbons) over the course of the experiment and a lack of statistical treatment of data hampered understanding relations between petroleum and the observed morphological changes.

Mutagenesis

The question of whether petroleum components are mutagenic and thus may threaten the viability of marine organisms exposed to discharges of these materials has received attention. For example, several studies examined the mutagenicity of crankcase oil. Fractions of used crankcase oil rich in polynuclear aromatic hydrocarbons produced revertant colonies of *Salmonella typhimurium* strain TA 98 (Ames test) (Payne et al. 1978b). The mutagenic activity represented by these changes was apparently not specifically linked to the known mutagens, benzo[a]pyrene or benz[a]anthracene. In another laboratory, the Ames test was adapted to the determination of the mutagenic properties of mineral oils (Hermann et al. 1980). Mutagenic activity was associated with used crankcase oils as well as with petroleum distillates; however, much less activity was found for solvent-refined oils that were low in aromatic hydrocarbons (Table 5). The mutagenic activities correlated well with the total aromatic hydrocarbon content of the oils rather than with specific polynuclear aromatic hydrocarbons (Hermann et al. 1979, 1980).

Data obtained thus far suggest that used crankcase oils and other petroleum products, which are released into the marine environment in substantial quantities, may represent a mutagenic burden to marine life; however, no field study exists to link directly petroleum in the environment to mutagenic changes in marine organisms or to related diseases, such as neoplasia.

In conclusion with respect to this section, the laboratory studies with petroleum and petroleum fractions support the findings from laboratory studies with individual hydrocarbons which imply that a variety of cellular and subcellular anomalies in marine organisms and their early developmental stages are related to exposure to petroleum components; however, it should again be strongly emphasized that many of the reported effects were brought about by environmentally unrealistic concentrations of petroleum or petroleum components.

Petroleum in the Marine Environment

A number of field studies, often taking advantage of oil spills, have attempted to determine whether cellular/subcellular changes occur in organisms exposed to petroleum in the marine environment. The large number of natural variables and a general lack of understanding of environmentally induced alterations in petroleum make the task of establishing cause-and-effect relations much more difficult than in laboratory studies.

In one field study, two species of flounder (winter flounder, *Pseudopleuronectes americanus*, and yellowtail flounder, *Limanda ferruginea*) were collected from control stations and from stations close to the 1976 ARGO MERCHANT oil spill; however, findings linking petroleum from the spill to morphological damage were not observed (Sawyer 1978). Studies of horse mussels (*Modiolus modiolus*) from the area of the ARGO MERCHANT spill did reveal certain gross alterations that were apparently related to petroleum. "White, round raised nodules, 1 to several mm in diameter" occurred in the overlying mantle where it was in contact with "spots of oil" (Brown and Cooper 1978). Another study of the ARGO MERCHANT spill suggested that abnormalities in chromosomes (arrested metaphase of mitosis) in the early embryo stage of pollock (*Pollachius virens*) resulted from exposure to the spilled oil (Longwell 1978). Embryos at all stations revealed some oil contamination of the chorion. Moreover, a station with the oil slick itself contained pollock embryos that were grossly malformed (18%), whereas at the periphery of the oil slick, fewer were affected (9%). No abnormalities were apparent in pollock embryos at any stations more distant from the oil slick than the one at the periphery, nor were any observed in cod embryos (Longwell 1978).

Studies of the 1978 AMOCO CADIZ oil spill off the Coast of France suggested a link between the spilled petroleum and gross pathological alterations, such as fin erosion in plaice (*Pleuronectes platessa*) from the Aber Benoit and the Aber Wrac'h (Miossec 1981).

A later study of plaice from the impacted Aber Benoit and Aber Wrac'h between 1978 and 1980 showed fin and tail necrosis, extensive gill lesions, abdominal muscle and gastric gland degeneration, and increased concentrations of hepatic macrophage centers (Haensly et al. 1981). A comparison of these findings to those from a reference area (Baie de Douarnenez and the ports of Loctudy and Ile Tudy) suggested a likely association between spilled oil and the observed biological alterations (Table 6).

A study of chronic exposure to oil (concentration not specified) indicated an apparent association between morphological changes in the liver of mummichogs and exposure to oil-contaminated waters off the East Coast of the United States (Sabo et al. 1975). Electron-microscopic examination of livers from contaminated *Fundulus* revealed proliferation of rough endoplasmic reticulum, coupled with decreased glycogen and lipid stores, and an increase in free ribosomes. The

TABLE 6. Observations on cellular changes of plaice, *Pleuronectes platessa*, from three sites along the Brittany coast over a 1½-yr period following the AMOCO CADIZ oil spill. (Abstracted from Haensly et al. 1981.)

Condition or measurement	Collection site	Collection Intervals: December 1978	April 1979	August 1979	February 1980	June 1980	Overall Mean
Percentage with fin and tail nercrosis on integument and integument appendages	Aber Wrac'h	NA[b]	60[c]	70	100	80	76
	Aber Benoit	0	40	80	100	100	62
	Reference[a]	0	10	0	10	20	9
Percentage with extensive lesions on the gills	Aber Wrac'h	NA	10[d]	20	0	0	8
	Aber Benoit	20	0	30	0	0	10
	Reference	0	0	0	0	10	2
Percentage with abdominal muscle fiber degeneration	Aber Wrac'h	NA	0	0	12	30	11
	Abenoit	20	0	10	0	40	15
	Reference	10	0	17	10	10	9
Percentage with gastric gland degeneration	Aber Wrac'h	NA	100	100	50	50	76
	Aber Benoit	90	90	80	75	80	83
	Reference	0	20	0	20	10	11
Percentage with dilation of Bowman's space in the kidney glomeruli	Aber Wrac'h	NA	90	78	38	100	78
	Aber Benoit	30	70	80	75	90	69
	Reference	70	40	33	70	0	43
Concentration of macrophage centers in the liver	Aber Wrac'h	NA	8.2[e]	3.6	10.2	8.3	7.4
	Aber Benoit	10.5	16.4	11.0	15.5	11.4	12.8
	Reference	3.6	0.5	1.3	9.5	6.9	4.6

[a]Reference sites were the Baie de Douarnenez and the ports of Loctudy and Ile Tudy.
[b]Not available. (No fish captured.)
[c]Percentage of fish with one or more lesions.
[d]Percentage of gills with lesions.
[e]Mean concentration of macrophage centers.

possible influence of various environmental factors (e.g. other xenobiotics) on the results were not addressed.

Field studies (Rinkevich and Loya 1979) showed that the average reproductive index recorded in corals (*S. pislillata*) from "oil-polluted" areas (near oil terminals) was significantly lower than for areas "free of oil pollution." These findings are generally consistent with the laboratory results of these workers, mentioned earlier in this review, which relate petroleum effects to gonadal development.

Gill damage (e.g. cell losses) and depletion of mucous cells were believed to be associated with spilled petroleum in a study of fish, e.g. flounder (*Ancyclopsetta quadrocellata*), caught off the Louisiana Coast, southeast of East Timbalier Island (Blanton and Robinson 1973); however, no data were presented on petroleum hydrocarbon concentrations in the environments or in the tissues of the species examined.

It has been suggested that neoplasia occurs in soft-shell clams obtained from petroleum-impacted sites along coastal areas of the United States. The neoplasms, found in organisms collected near Long Cove, Searsport, and Harpswell Neck in Maine, were present in the reproductive tract and gonads (Yevich and Barszcz 1976, 1977; Barry and Yevich 1975). Animals having gonadal neoplasms showed a high incidence of metastasis, especially to the gills. Later studies by Brown (1980) and Oprandy et al. (1981) suggested that a virus infection was actually responsible for the neoplasms in the soft-shell clams. Exposure of an estuarine bryozoan (*Schizoporella unicornis*) to coal tar derivatives in the field (Powell et al. 1970) induced hyperplasia; however, other causative agents in the environment may have similarly contributed to this pathological condition.

Oysters (mainly *Juncus roemerianus*) exposed to 4 mg/L of Empire Mix, and Saudi Arabian and Nigerian crude oils in estuarine ponds showed alterations in connective tissues associated with the body wall, mantle, and food groove (Barszcz et al. 1978). Connective tissue fibers of muscle in these areas "were swollen, translucent, homogeneous, structure-less masses" which closely resembled the condition usually described as hyaline degeneration (a condition believed to be the result of an interference in protein metabolism). Moreover, a reduced reproductive potential was indicated by the fact that there was a decrease in the development of germinal epithelial tissues (Barszcz et al. 1978).

Cockles (*Cerastoderma edule*) and mussels (*Mytilus edulis*) obtained from Presqu'ile Quiberon were held in cages at a depth of 1 m in the Baie de Morlaix, a site known to have been heavily oiled by the AMOCO CADIZ, and the Rade de Brest, a site known to be minimally exposed, from April 27 to May 22, 1978 (Wolfe et al. 1981). Organisms were sampled 3 or 4 times over 25 d for histological examination and analysis of hydrocarbons. Body burdens of hydrocarbons and polar aromatic compounds (possibly metabolites) increased in both molluscan species notably at the Baie de Morlaix site. At the Morlaix site, up to five-fold increases in hydrocarbon content had occurred within 5 d after transplantation, and these concentrations were maintained (or decreased slightly) throughout the sampling period. Organisms from the Rade de Brest accumulated hydrocarbons to about the same levels.

TABLE 7. Examples of biological effects arising from exposing marine organisms in the laboratory to relatively low concentrations of petroleum and petroleum components.

Type of organism	Type of petroleum or petroleum component/ mode of exposure	Concn. (exposure period)	Biological effects	Reference
Teleosts				
Atlantic silversides (*Menidia menidia*)	Louisiana crude oil/ in seawater	140 μg/L (168 h)	Hyperplasia of the olfactory sustentacular epithelium; severe degeneration of olfactory mucosa	Gardner 1975
English sole (*Parophrys vetulus*)	Prudhoe Bay crude oil/ in sediment	0.2–1.0% v/v (0.75–4 mo)	Severe hepatocellular lipid vacuolization; wt loss; alterations in hemoglobin and hematocrit values	McCain and Malins 1982
Mummichogs (*Fundulus heteroclitus*)	Naphthalene/ dissolved in salt water	200 μg/L (15 d)	Ischemia in brain, liver, and pancreas; lateral line necrosis; elevation in serum glucose, protein, and cortisol	DiMichele and Taylor 1978
Pacific herring (*Clupea harengus pallasi*)	Benzene/ dissolved in seawater	0.9 μg/L (24 h)	Delayed embryonic development	Eldridge et al. 1977
Pacific herring (*Clupea harengus pallasi*) embryos	Prudhoe Bay crude oil/ in seawater	<1 μg/g (2–6 d)	Gross morphological abnormalities (e.g. failure of fins to differentiate from the lateral line body wall)	Smith and Cameron 1979
Surf smelt (*Hypomesus pretiosus*) embryos	Cook Inlet crude oil/ accommodated in seawater	54 and 113 μg/L 3 h/d for 15 and 21 d)	Necrotic neurons in the forebrain and the neuronal layer of the retina	Hawkes and Stehr 1982
Invertebrates				
Clams (*Mercenaria mercenaria*)	Phenol/ in seawater	100 μg/L (24 h)	Cytological damage in gill, digestive gland, and hindgut	Fries and Tripp 1977
Lobsters (Homarus *americanus*)	South Louisiana crude oil/ in seawater	100 and 1000 μg/L (15 d)	Distended chromatophores	Forns 1977
Mussels (*Mytilus edulis*)	Aromatic hydrocarbons from North Sea crude oil/ in seawater	30 μg/L (34–182 d)	Reduction in heights of digestive cells; structural changes in secondary lysosomes	Lowe et al. 1981
Sea urchin eggs (*Paracentrotus lividus*)	Benzo[a]pyrene/ in seawater	4–5 μg/L (3 h)	Abnormal cleavage in embryos; development into irregular morulae	Ceas 1974

Digestive diverticula from mussels in the Baie de Morlaix had much larger accumulations of lipid and lysosomal granules than comparable tissue from mussels in the Rade de Brest. An increased number of lipid droplets were evident in the cells of the primary and secondary ducts as well as in the blind-ending tubules, whereas increases in lysosomal granules were restricted to the latter. Within the tubules, absorptive cells were more affected than the basophilic secretory cells.

Cockles from Baie de Morlaix also showed increased number of lipid droplets and lysosomal granules in digestive gland cells, and there were clear histological differences detected between mussels from each site, despite the fact that significant differences did not exist in the body burdens of hydrocarbons (Wolfe et al. 1981). No lesions were observed in gill tissue of either species. The cytological changes were not necessarily an example of pathological changes, although they may reflect a chemically induced stress response. It is of interest that the lysosomal changes observed in the field studies are generally consistent with findings from previously mentioned laboratory studies (Lowe et al. 1981; Moore et al. 1978a, 1980).

At Roscoff, a number of pathological alterations were found in eels (species not given) after contamination with oil from the AMOCO CADIZ (Lopez et al. 1981). Gills showed abnormal proliferation of ionocytes (chloride cells) and of mucous cells, and hypersecretion of mucus correlated with hyperemia and a lysis of respiratory platelets. Basophilic ovarian intracytoplasmic inclusions were thought to be related to hydrocarbon accumulations. Necrosis of follicles was enhanced after 8 mo and resulted in a complete degeneration

of oocytes. A substantial stimulation of interrenal activity was also noted (glandular hypertrophy, increased plasma cortisol, and increased biosynthesis of corticosteroids by renal slices).

In summary, the studies of the effects of AMOCO CADIZ oil on marine life indicated that a variety of deleterious cellular/subcellular alterations were associated with this massive oil spill. Nevertheless, little generic information was obtained to facilitate an understanding of how, or under what circumstances, such morphological changes are linked to petroleum exposures in other marine environments.

Conclusions

Laboratory studies have shown that individual aromatic hydrocarbons, whole oils, and petroleum fractions are capable of inducing, under certain conditions, a variety of morphological alterations in exposed marine organisms. Examples of such studies are given in Table 7 in which relatively low concentrations of petroleum were used. Although the findings are clearly advances in knowledge, little progress has been made in delineating the significance of observed morphological changes. What, for example, is the impact of currently observed morphological changes on marine ecosystems and the health and survival of organisms? In my opinion, too few credible studies have been conducted thus far with different types of organisms to answer cogently these important questions.

Additional problems exist. No real understanding has actually been obtained about how the petroleum-induced changes relate to the degree or time of exposure, or to a variety of other experimental conditions. Perhaps the most obvious problem with most of the laboratory studies was the use of exposure concentrations that were often orders of magnitude higher than those reported to occur in the marine environment.

More information is clearly needed about the sensitivities of different species to petroleum-induced morphological alterations. Moreover, there is an increasing awareness that early developmental stages (embryos and larvae) are particularly susceptible to deleterious effects at the cellular/subcellular level, sometimes at exposure concentrations that are substantially lower than those inducing morphological changes in juvenile or mature organisms. Most of the relevant information on these early life stages has been derived from laboratory studies. The paucity of information on effects in the field is due to many factors, although it may be partly attributed to the fact that impacted early developmental stages do not survive long in marine environments.

Field studies, notably those conducted in relation to the AMOCO CADIZ incident, have indeed shown that gross pathological and cellular/subcellular alterations in marine organisms are associated with spilled petroleum; however, the data have little quantitative significance with regard to effects on marine resources. In addition, certain observed morphological changes (e.g. hepatocellular lipid vacuolization in marine fish) (McCain et al. 1978), which appear to be reversible, are questionable indicators of altered organism viability. Moreover, the relationship between chemically (e.g. light-catalyzed oxidations of aromatic hydrocarbons) and biologically (e.g. metabolism of hydrocarbons) induced changes (Malins 1980) in petroleum components in the field and deleterious effects at the cellular/subcellular level remain largely unknown. Thus, this important question cannot at present be adequately addressed: "Are environmental transformations in petroleum components enhancing or retarding deleterious morphological changes associated with petroleum exposure?"

It is recognized that petroleum components exist together with myriad other potentially toxic compounds in most urban-associated coastal marine environments (Malins et al. 1980) and that synergism and antagonism influence biological changes in marine organisms (Malins and Collier 1981). Accordingly, serious doubts are raised about claims that cellular/subcellular alterations in organisms from "petroleum impacted" areas are specifically related to petroleum components or their transformation products. On some occasions, confusion also exists about whether petroleum directly brings about morphological changes in organisms exposed in the field or whether the effect is indirect, such as through the influence of pathogens (e.g. viruses), or through interactions between complex mixtures of natural and man-made chemicals.

In summary, petroleum (generally at higher concentrations than found in marine environments) has been shown to be definitely associated with a number of potentially deleterious cellular and subcellular alterations in marine organisms exposed in the laboratory and field; however, in the marine environment predictions about the impact of petroleum exposure on organisms have been difficult to make because of the influence of myriad factors that are in a continual state of flux. In this regard, studies that explore the possibility of synergism and antagonism between petroleum components and other pollutants are recommended as a means of broadening our understanding of such problems (Malins and Collier 1981).

A clear need exists for conducting carefully controlled field experiments that relate environmental exposures of petroleum to alterations in cellular and subcellular structure, as well as to a variety of biochemical and physiological changes. In addition, it is suggested that currently available techniques of histology and clinical chemistry have not been adequately exploited in efforts to link petroleum in marine environments to changes in the viability of organisms and ecosystems. In future work, the appropriate use of statistical methods in relating petroleum exposure to biological changes needs to be emphasized.

It is also important to stress that future emphasis should be placed on conducting scientifically credible laboratory studies that reflect concentrations of petroleum and other conditions that are realistic for marine environments and thus serve as a basis for interpreting data from the field. Overall, the failure to do this so far has severely restricted the application of laboratory studies, in particular, to understanding the morphological effects of petroleum on marine life.

Acknowledgments

Appreciation is expressed to the following individuals for their comments on the manuscript: Dr Harold Hodgins, Dr Sin-Lam Chan, Dr Joyce Hawkes, Dr Edmundo Casillas, Dr James Bruya, Dr William T. Roubal, Mr Tracy Collier, and Mr Paul Robisch.

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Aquatic Invertebrates from the Smoking Hills, N.W.T.: Effect of pH and Metals on Mortality

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HAVAS, M., AND T. C. HUTCHINSON. 1982. Aquatic invertebrates from the Smoking Hills, N.W.T.: effect of pH and metals on mortality. Can. J. Fish. Aquat. Sci. 39: 890–903.

Experiments were conducted on planktonic crustaceans and insect larvae from acidic and alkaline tundra ponds at the Smoking Hills, N.W.T. to determine their tolerance to low pH and elevated levels of potentially toxic elements, including Al. The crustaceans (*Daphnia middendorffiana, Diaptomus arcticus, Lepidurus arcticus, Branchinecta paludosa*), which are found only in alkaline ponds, died rapidly below pH 4.5. The insect larvae (*Orthocladius consobrinus* and *Limnephilus pallens*) from the same alkaline ponds were able to survive for extended periods at pH 3.5, though they do not occur in acidic waters at the Smoking Hills. The red chironomid (*Chironomus riparius*) is restricted to acidic ponds although it was able to survive not only at pH 2.8, but also in pond water of pH 8.2.

Water from an acidic pond (pH 2.8) was markedly more toxic to crustaceans than water from an alkaline pond (pH 8.2) when both were adjusted to pH 4.5. Elevated concentrations of aluminum may account for this additional toxicity of the acidic pond water. Levels reached 20 mg/L Al, and in experiments with Al, additions to the alkaline waters and to the acidic pond waters, after metal removal, caused toxicity to the crustaceans.

The absence of crustaceans from acidic ponds at the Smoking Hills may be due to their extreme sensitivity to low pH. The similarly restricted distribution of certain of the insect larvae, in contrast, cannot be explained this simply. Metal concentrations in acidic ponds impose an added stress. Aluminum was found to be the key additional factor to that of H^+ ion concentration.

Key words: acidic ponds, tundra ponds, Smoking Hills, bioassays, zooplankton, insect larvae, crustaceans, *Daphnia*, *Diaptomus*, *Lepidurus*, *Brachinecta*, *Chironomus*, *Orthocladius*, *Limnephilus*, heavy metals, aluminum, calcium, sodium, iron, zinc, nickel

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Des expériences ont été menées sur des crustacés et des larves d'insectes provenant d'étangs de toundra acides et alcalins des collines Smoking (T.N.-O.) dans le but de déterminer leur tolérance à un bas pH et à des teneurs élevés en éléments potentiellement toxiques, y compris Al. Les crustacés (*Daphnia middendorffiana, Diaptomus arcticus, Lepidurus arcticus, Branchinecta paludosa*), rencontrés seulement dans les étangs alcalins, meurent rapidement à un pH inférieur à 4,5. Les larves d'insectes (*Orthocladius consobrinus* et *Limnephilus pallens*) des mêmes étangs alcalins survivent de longues périodes à un pH de 3,5, bien qu'on ne les trouve pas dans les eaux acides dans les collines Smoking. Le chironomide rouge (*Chironomus riparius*) ne se rencontre que dans les étangs acides, bien qu'il puisse survivre non seulement à un pH de 2,8, mais aussi dans des eaux de pH 8,2.

L'eau d'un étang acide (pH 2,8) est nettement plus toxique pour les crustacés que celle d'un étang alcalin (pH 8,2) quand les deux sont ajustés à un pH de 4,5. Il se peut que de hautes teneurs en aluminium soit responsables de cette toxicité supplémentaire de l'eau d'étang acide. Les niveaux d'aluminium peuvent atteindre 20 mg/L, et dans des expériences au cours desquelles Al est ajouté à des eaux alcalines et acides,après enlèvement desmétaux, on observe des mortalités de crustacés.

L'absence de crustacés dans les étangs acides des collines Smoking peut être due à une sensibilité extrême à un bas pH. Par contre, on ne peut expliquer aussi simplement une

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Printed in Canada (J6171)
Imprimé au Canada (J6171)

distribution également limitée de certaines larves d'insectes. Les concentrations de métaux dans les étangs acides représentent une contrainte supplémentaire. En plus de la concentration de H^+, on constate que l'aluminium est le facteur-clé supplémentaire.

Received July 24, 1980
Accepted March 3, 1982

Reçu le 24 juillet 1980
Accepté le 3 mars 1982

THE widespread regional acidification of lakes and streams is a relatively recent phenomenon, but one which is spreading and accelerating (Oden and Ahl 1970; Likens et al. 1972; Beamish 1974; Grahn et al. 1974; Braekke 1976). A major concern is that acidified lakes are unable to support a diverse flora and fauna. The decline of fish populations, attributed to acid precipitation in the early 1960s, was first reported in Norway (cited in Leivestad et al. 1976). Similar trends have been observed in Sweden, the United States, and Canada (Beamish and Harvey 1972; Dickson 1975; Schofield 1976). While attention has focused primarily on fish, studies have also shown that phytoplankton, zooplankton, and benthic invertebrates have also decreased in diversity in recently acidified waters (Sutcliffe and Carrick 1973; Almer et al. 1974; Sprules 1975a, b; Hendrey et al. 1976; Leivestad et al. 1976).

There is ample evidence to suggest that pH is a major variable in determining the distribution of species. For example, gastropods are very sensitive to low pH (Okland 1969), as are blue-green algae (Brock 1973). The alga *Euglena mutabilis*, in contrast, is only found in waters of extreme acidity (Lackey 1938; Whitton 1975).

In laboratory experiments there is a critical pH, below which survival is significantly reduced for some species (Leivestad et al. 1976). Critical values for many fish, zooplankton, and gastropods lie in the pH range 5–6. The specific threshold values are a function not only of the species, but also of the chemical composition of the water. The concentration of calcium, sodium, carbon dioxide, and heavy metals can markedly influence the rate of mortality at low pH (Lloyd and Jordan 1964; Davis and Ozburn 1969; Sprague 1975; Leivestad et al. 1976).

In addition to man-induced acidification, naturally acidified waters also occur and can provide valuable information on potential biological responses to acid stress. Naturally acidified environments include bog lakes, volcanic lakes, thermal springs, and runoff from shale deposits.

Hutchinson et al. (1978) and Havas (1980) have studied the effect on tundra ponds of sulfur fumigations arising from spontaneously burning bituminous shale deposits in seacliffs at the Smoking Hills, N.W.T. Large amounts of sulfur dioxide released during the combustion of the shales have resulted in the acidification of the surrounding tundra soils and ponds. Resultant pHs of these ponds range from 1.8 near the fumigation to 9.7 in nearby non-fumigated areas. The chemical composition of the water, as well as the biological diversity, has been altered significantly by these fumigations. While species diversity is decreased in the acid ponds, the survival of rotifers, chironomids, and some algae, in ponds with extreme acidity (pH 1.8–2.8) and very high levels of Al, Mn, Fe, and Zn, suggests a significant adaptation. Indeed, the flora and fauna of the ponds of pH >5.0 and <4.5 are quite distinct, despite their close proximity.

The purpose of the present study was to assess the relative tolerance to a wide range of pH of selected aquatic invertebrates from the highly acidic and alkaline ponds at the Smoking Hills, and to determine if some of the species from alkaline waters had tolerance to acidic conditions. In addition to pH tolerance tests, the relative effect of some metals and bases (Al, Fe, Ni, Zn, Ca, and Na) on short-term survival of aquatic invertebrates was also assessed. The ability of these organisms to maintain their body burdens of osmotically important elements, such as Na, K, Cl, and Ca, when subjected to these stresses, was evaluated at the same time and is the subject of a separate report (Havas and Hutchinson 1982).

Methods

Bioassays were conducted in a simple field laboratory at Tuktoyaktuk, N.W.T., and in ponds at the Smoking Hills in 1976, 1977, and 1978 to determine the effect of pH on mortality of aquatic invertebrates. Both the water and the biota, used in these experiments, were collected either from a non-fumigated, alkaline pond (pH 8.2), or from a heavily fumigated, acidic pond (pH 2.8). The chemistry of these two ponds is summarized in Table 1. For brevity these ponds are designated 'control' and 'fumigated' as the former represents an area unaffected by fumigations.

Effect of pH on Mortality: Laboratory Bioassays

Seven species of aquatic invertebrates, common at the Smoking Hills sites, were used in the laboratory experiments. These include four crustaceans: *Daphnia middendorffiana*, *Diaptomus arcticus*, *Lepidurus arcticus* (tadpole shrimp), and *Branchinecta paludosa* (fairy shrimp); and three insect larvae: *Limnephilus pallens* (Trichoptera), *Orthocladius* (*Pogonocladius*) *consobrinus* (Diptera), and *Chironomus riparius* (Diptera). All of the species were collected from the control (alkaline) pond, except *C. riparius* which was collected from the fumigated (acidic) pond. This is simply a function of their occurrence.

The water used in the bioassays was filtered through a Whatman 42 filter to remove zooplankton and large suspended particles. pH was adjusted using Analar 0.05 mol/L H_2SO_4 and NaOH. pH-adjusted water was allowed to 'age' from 6 to 24 h (depending on sample volume) prior to experimentation to reduce the CO_2 concentrations in the acidified samples.

Individuals were placed into 21-mL Rigo vials containing 20 mL pond water adjusted from pH 2.8 to 8.2. Because the size and abundance of individual test species differed, the number of replicates and experimental densities varied accordingly (see Table 2 for details). Vials were checked daily for mortality (more frequently at the start of the experiment). Minor shifts in pH were adjusted daily and seldom exceeded 0.2 pH units. Vials were exposed to 24 h of daylight. Temperatures during the experiment fluctuated from 12 to 18°C.

TABLE 1. Chemical composition of pond water used in the laboratory and field bioassays.

Analysis	Units	Pond water			
		Control		Fumigated	
pH	$-\log [H^+]$	8.2		2.8	
Specific conductivity	$S \cdot cm^{-1}$ 25°C	2.94		1.30	
Alkalinity	mmol $CaCO_3 \cdot L^{-1}$	1.9	(95)[a]	0	(0)
Acidity	mmol $CaCO_3 \cdot L^{-1}$	0	(0)	6.4	(320)
Dissolved oxygen	mmol $\cdot L^{-1}$	0.31	(9.9)	0.30	(9.5)
Organic carbon	mmol $CO_2 \cdot L^{-1}$	0.43	(19)	0.11	(5)
Inorganic carbon	mmol $CO_2 \cdot L^{-1}$	0.70	(31)	0.05	(2)
SO_4	mmol $\cdot L^{-1}$	7.96	(765)	2.87	(276)
Cl	mmol $\cdot L^{-1}$	8.97	(318)	0.51	(18)
HCO_3	mmol $\cdot L^{-1}$	1.22	(71)	0	(0)
NO_3	μmol $\cdot L^{-1}$	7.1	(0.44)	194	(12)
PO_4	μmol $\cdot L^{-1}$	0.23	(0.022)	0.37	(0.035)
Na	mmol $\cdot L^{-1}$	8.22	(189)	0.91	(21)
Ca	mmol $\cdot L^{-1}$	6.29	(252)	1.12	(45)
Mg	mmol $\cdot L^{-1}$	6.21	(151)	0.82	(20)
Al	μmol $\cdot L^{-1}$	<26.	(<0.7)	667	(18)
Fe	"	2.1	(0.12)	501	(28)
Mn	"	<5.5	(<0.3)	51.0	(2.8)
Zn	"	0.153	(0.01)	16.8	(1.1)
Ni	"	0.085	(0.005)	3.44	(0.202)
Cd	"	<0.036	(<0.004)	0.489	(0.055)
U	"	<0.042	(<0.010)	0.15	(0.036)
As	"	<0.093	(<0.007)	0.04	(0.003)

[a]Concentrations in mg/L are presented in parentheses.

EFFECT OF pH ON MORTALITY: POND BIOASSAYS (IN SITU STUDY)

Bioassays were conducted in 1977 in one control and one fumigated pond using Plexiglas enclosures to isolate water columns. Plexiglas of 0.64 cm ($\frac{1}{4}$ in.) thickness was used to construct rectangular columns measuring 0.61 m × 0.61 m × 1.22 m. Three enclosures were anchored in the sediment of each of the two experimental ponds. During July 1977, the active layer in the sediment had thawed to a depth of 20–30 cm. The water level was 20 cm below the top of the Plexiglas enclosure and was thus sufficient to prevent mixing with the rest of the pond on windy days.

The pH of the water in the enclosures was adjusted to 3.0, 4.5, and 8.0 using concentrated H_2SO_4 and NaOH. The CO_2 in the water was allowed to escape into the atmosphere after acidification for $2\frac{1}{2}$ d prior to experimentation. Total inorganic carbon decreased from 25 mg^{-1} (pH 8.2) to 3.5 mg (pH 4.5) and to 1 mg^{-1} (pH 3.0). Minor adjustments in pH (less than 0.5 pH units) were made throughout the experiment.

'Cages' for the experimental exposure of the biota were constructed using 52-mL (14 dram) Rigo vials. Large holes in the lid and bottom of the vials were covered with Nitex netting. Mesh size was 656 μm for *Daphnia*, *Diaptomus*, and the two diptera larvae, and 1050 μm for *Branchinecta* and *Limnephilus*. This allowed the water to move freely between the cages and the surrounding water. Individuals were placed into the cages, which were suspended in the cylinders midway between the sediment and the water surface, with fishing line fastened to wooden frames on top of the cylinders.

Mortality was recorded daily (more frequently at the start of the experiment). Temperatures fluctuated between 3 and 6°C. Vials were exposed to 24 h of daylight.

EFFECT OF THE METALS, AL, FE, NI, ZN, AND CA ON MORTALITY OF *DAPHNIA*

Two approaches were used to determine the effect of Ca, Na, and selected 'heavy' metals on mortality of *Daphnia middendorffiana* at pH 4.5.

The first approach was to alter the fumigated pond water. Thus, (1) calcium (as $CaCl_2$) was added to raise the water-Ca concentration from 45 to 250 mg/L, which corresponded to the natural concentration in the control pond water (Table 1). Similarly, (2) Na (as NaCl) was used to increase the Na concentration from 20 to 200 mg/L. A further amendment for the fumigated pond water involved (3) reducing the concentrations of heavy metals. This was accomplished by raising the pH to 8 with NaOH, and then filtering the resultant precipitate through a Whatman 42 filter, followed by a 0.45-μm Millipore filter. Even those metals that become soluble at high pH decreased in concentration as a result of coprecipitation with Fe. This technique did not significantly alter the Ca concentration as shown by analyses, but effectively eliminated Al, Fe, Mn, Zn, and Ni. All experiments were conducted at pH 4.5.

The second approach involved amendments to the non-fumigated control pond water. Four potentially toxic metals, which are readily leached from soils and sediments exposed to acid precipitation and which were found to be substantially elevated in the fumigated pond water (Table 1) were used (Ni, Zn, Al, Fe). Chloride salts of these metals were added to the

control pond water to achieve the concentrations which occurred in the fumigated pond water (i.e. Ni 0.2 mg/L, Zn 1.2 mg/L, Al 20 mg/L, Fe 30 mg/L (Table 1). By determining survival in waters with additions of each of these elements individually, we hoped to assess their respective roles in influencing mortality.

Experiments were conducted in 52-mL Rigo vials at pH 4.5 using *D. middendorffiana*. Daphnids were exposed to the different treatments described above. Mortality was recorded daily. Results are based on three replicate vials, each containing 10 individuals.

Chemical Analysis of the Water

Water samples were collected for chemical analyses at the start of each laboratory experiment and also midway through the in situ experiment. Water samples for metal analyses were Millipore-filtered (0.45 μm), acidified with concentrated Analar grade HNO_3, and stored in acid-washed Nalgene bottles. Iron, Zn, Ni, and Cd were analyzed by Atomic Absorption Spectroscopy (AAS) (Perkin–Elmer 460) with an air–acetylene flame and a single hollow cathode lamp as the light source. A graphite furnace (Perkin–Elmer 2100) was used for the determination of Cd and Ni at low concentrations. Sodium, Ca, Mg, Al, Mn, U, V, and Cl were determined by Instrumental Neutron Activation Analysis (INAA) using the SLOWPOKE reactor at the University of Toronto.

Additional water samples were collected on day 1 of the in situ experiment to determine total inorganic and organic carbon. These were preserved with HgCl in 2-mL ampules which were heatsealed immediately following collection. Determinations were made using an infrared Total Organic Analyzer (Beckman model 915).

Standards were prepared from analytically pure reagents or from Fisher's Certified Atomic Absorption 1000 mg/L stock solutions diluted with 5% Analar grade HNO_3. Details of the graphite furnace AAS, INAA, and the carbon analyses are provided in Havas (1980).

Statistical Analysis

The Kolmogorov–Smirnov test is a nonparametric test used to determine significant differences between two continuously distributed curves (Sokal and Rohlf 1969; Campbell 1974). It was used in these experiments to determine between mortality curves. Results are shown in Fig. 4.

Results and Discussion

Crustacean Responses

All crustaceans were very sensitive to low pH. Responses under field and laboratoy conditions were very similar. *Daphnia middendorffiana*, *Diaptomus arcticus*, *L. arcticus*, and *B. paludosa* were all uniformly sensitive to low pH. In non-fumigated pond water adjusted to pH 3.5 and 4.0, all individuals of all four species died within 12 h (Fig. 1–3). The critical pH (i.e. the pH below which the rate of mortality increases significantly compared to the control) was around pH 4.5. Mortality was significantly delayed at this pH ($P < 0.01$) compared with lower pHs (Fig. 4). There was no

TABLE 2. Experimental details of laboratory and pond bioassays. Experiments were conducted in "control" and "fumigated" pond water

	Laboratory bioassay (July and August, 1976)							Pond bioassay (in situ) (July 1977)					
	pH				Organisms per vial[a]	Replicates		pH			Organisms per cage[b]	Replicates	
Species													
Crustacea													
Daphnia middendorffiana	3.5	4.0	4.5	5.5	8.2	10	3	3.0	4.5	8.0	10		5
Diaptomus arcticus	3.5	4.0	4.5	5.5	8.2	10	3	3.0	4.5	8.0	10		5
Lepidurus arcticus	3.5		5.5		8.2	1	6		—		—		—
Branchinecta paludosa	3.5	4.5		5.5	8.2	1	5	3.0	4.5	8.0	5		5
Insecta													
Limnephilus pallens	2.8	3.5	4.5	5.5	8.2	1	5	3.0	4.5	8.0[c]	1		10
Orthocladius consobrinus	2.8	3.5	4.5	5.5	8.2	10	3	3.0	4.5	8.0	5		5
Chironomus riparius	2.8	3.5	4.5	5.5	8.2	6	3	3.0	4.5	8.0	5		5

[a]Size of vial, 21 mL.
[b]Size of cage, 52 mL.
[c]Experiment conducted only in the control water.

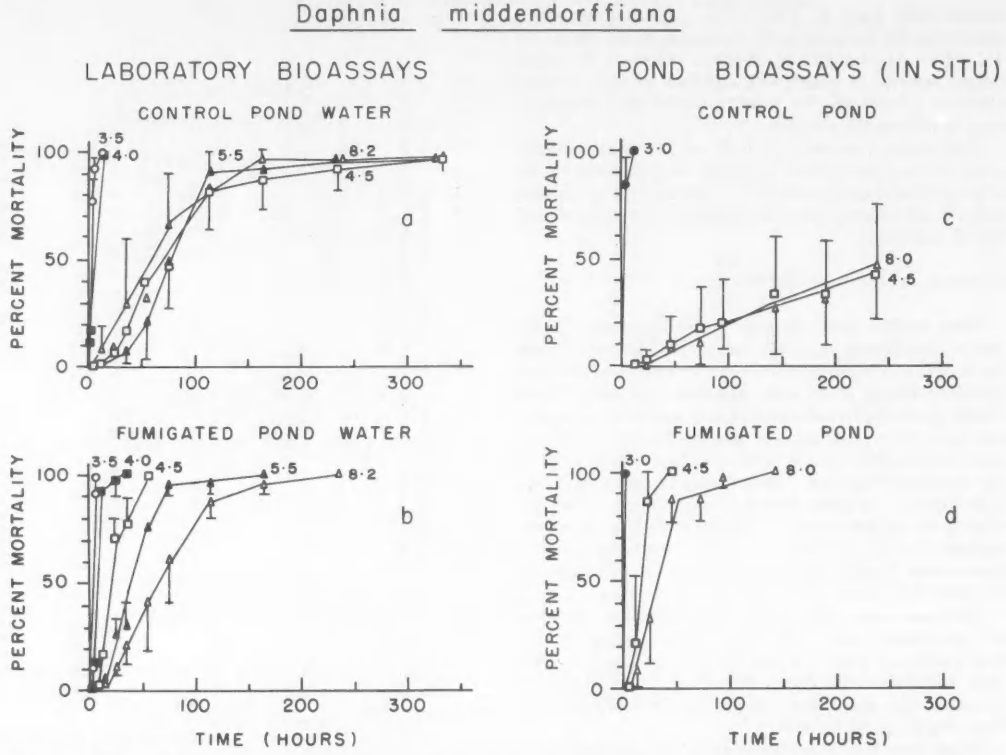


FIG. 1. Mortality curves for *Daphnia middendorffiana* at different pHs in laboratory and pond bioassays. Numbers represent experimental pH. Vertical bars represent 1 SD.

statistical difference in the mortality curves for pH 4.5, 5.5, and 8.2 in non-fumigated water in either the laboratory or pond experiments (Fig 4). In fumigated pond water the patterns of mortality observed in the 'control' water were repeated, though toxicity was enhanced, especially at pH 4.5. This is especially clear in the pond bioassays when Fig. 1–3 are examined.

The sensitivity of crustaceans to low pH is not surprising as all the species tested in these experiments were collected from an alkaline pond. The critical pH for survival of the crustaceans (pH 4.5) in these experiments was slightly lower than the values quoted in the literature. Sprules (1975b) examined 47 lakes in the La Cloche Mountains of Ontario and found that 64% of the zooplankton species were never or rarely found below pH 5.0. Similarly, in Norway, *Daphnia galeata*, *Ancanthodiaptomus denticoruis*, *Heterocope appendiculata*, and *Cyclops* spp. were absent from lakes at pH $\leq$ 5.0 (Hendrey and Wright 1975). Neither *Daphnia* (Almer et al. 1974) nor *Gammarus* (Hendrey and Wright 1975) are common below pH 6 in Scandinavia. The experimental findings of Leivestad et al. (1976) suggests that pH 5.5 is critical for the survival of *L. arcticus* and *Gammarus lacustris*. The delay in molting at pH 5.5, which they noted, was also observed in our experiments for *L. arcticus*. One probably reason that the critical pH in our experiments is lower than those values reported in the literature is that the Smoking Hills ponds are hardwater bodies rather than softwater lakes. The calcium concentration in these ponds is high (250 mg Ca/L). Calcium has been shown to ameliorate H^+ toxicity (Lloyd and Jordan 1964; Davis and Ozburn 1969) and also Al toxicity.

Two different types of water were used in these experiments. Mortality of the crustaceans at pH 4.5 was significantly more rapid in the fumigated pond water than in the 'control' pond water, $P \leq 0.01$, Fig. 1–4. Factors additional to that of pH must have affected mortality in fumigated pond water. Differences in water chemistry between the ponds were considered in subsequent experiments.

The fairy shrimp was extremely sensitive to handling, so that it presented difficulties as an experimental animal. Although mortality was more rapid at the lower pHs, it was highly variable under control conditions in the laboratory experiments (Fig. 3a and b). In the field experiments in the 'control' pond, predation by *Orthocladius* in the cages interfered with the results. Despite this, Fig. 3c and d show that mortality was much more rapid at pH 4.5 in the fumigated water compared with the control water.

Chemical analysis of the water from control and fumigated ponds shows several major differences. Concentrations of

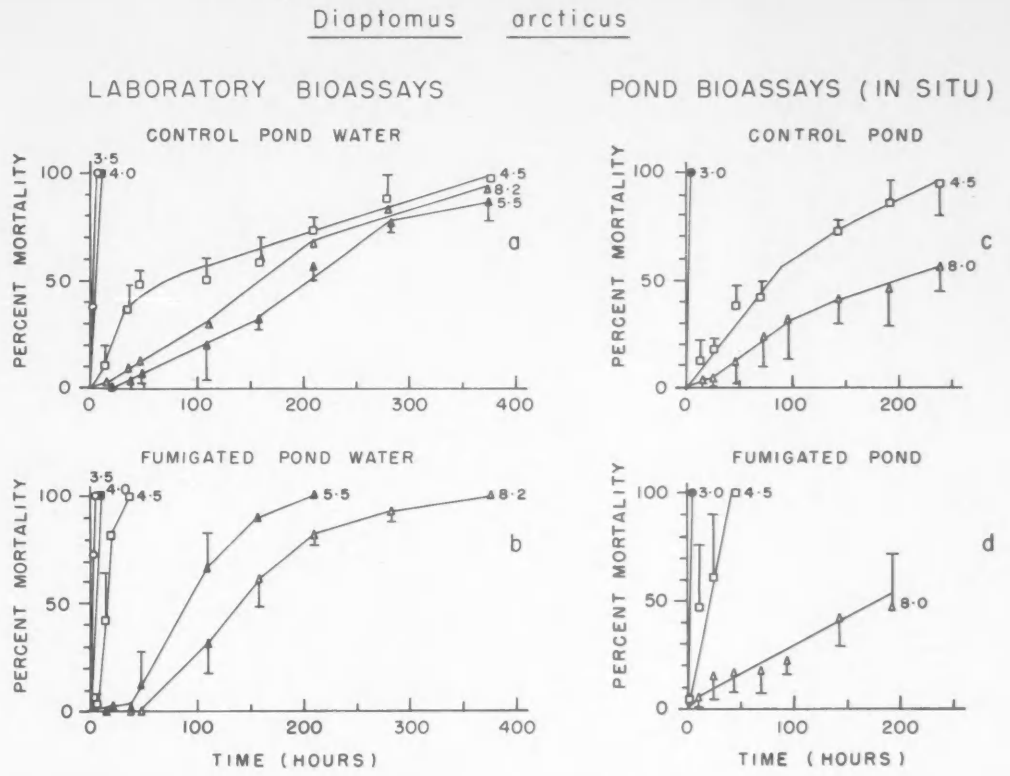


FIG. 2. Mortality curves for *Diaptomus arcticus* at different pHs in laboratory and pond bioassays. Numbers represent experimental pH, vertical bars represent 1 SD.

calcium, magnesium, sodium, and chloride were between 5 and 10 times higher in the control pond water, while the concentrations of toxic metals such as Al, Fe, Mn, Zn, and Ni were significantly elevated in the fumigated pond water (Table 1). All of the heavy metals (i.e. Fe, Al, Mn, Ni, Zn, Cd, V, and U) decreased as the pH of the fumigated water was raised experimentally (Fig. 5 and 6). The overall concentration of these metals was so high that during titration, as the pH approached 4.0, a thick orange precipitate formed. This consisted primarily of iron and aluminum hydroxides. In the field bioassays in the fumigated pond, when the water was raised to pH 8, an orange flocculant ~0.5 cm deep covered the sediments.

The solubility of elements is pH dependent (Stumm and Morgan 1970). It has been shown that the concentration of Fe and Al decrease exponentially at pH greater than 4.0 (Campbell et al. 1964). Other elements such as Ni, whose solubility is not as pH dependent, decreased in concentration at higher pHs as a result of co-precipitation.

Cl and Ca concentrations in the fumigated pond remained uniformly low following pH adjustments, while Na increased slightly at higher pH as a result of the addition of NaOH (Fig. 5).

In the control water, pH adjustments had little effect on water chemistry. Na, Cl, Ca, and total organic carbon concentrations remained uniformly high, while Fe, Al, Mn, Ni, Zn, Cd, V, and U remained uniformly low in both the laboratory (Fig. 5) and the field study (Fig. 6). The only significant pH-related change occurred for total inorganic carbon, which decreased from 25 mg/L at pH 8 to 3.5 mg/L at pH 4.5 and to 1 mg/L at pH 3. The loss of inorganic carbon during acidification was due to a change from HCO_3^- to CO_2, which is rapidly lost from the water as it equilibrates with the atmosphere.

The only other change that occurred in the control pond in the field experiments was a slight increase in the concentrations of Mn, Fe, and Cd at the lower pHs. Solubilization of heavy metals from soils and sediments under acid conditions is well documented (Whitby and Hutchinson 1974). The sediment enclosed by the Plexiglas cylinder was the probable source of this increase in the acidification experiments.

While numerous differences other than pH occurred in the chemistry of the two ponds, differences in mortality at pH 4.5 were hypothesized to be due to either toxicities or deficiencies of key elements.

The effect of Ca was examined because it is reported that

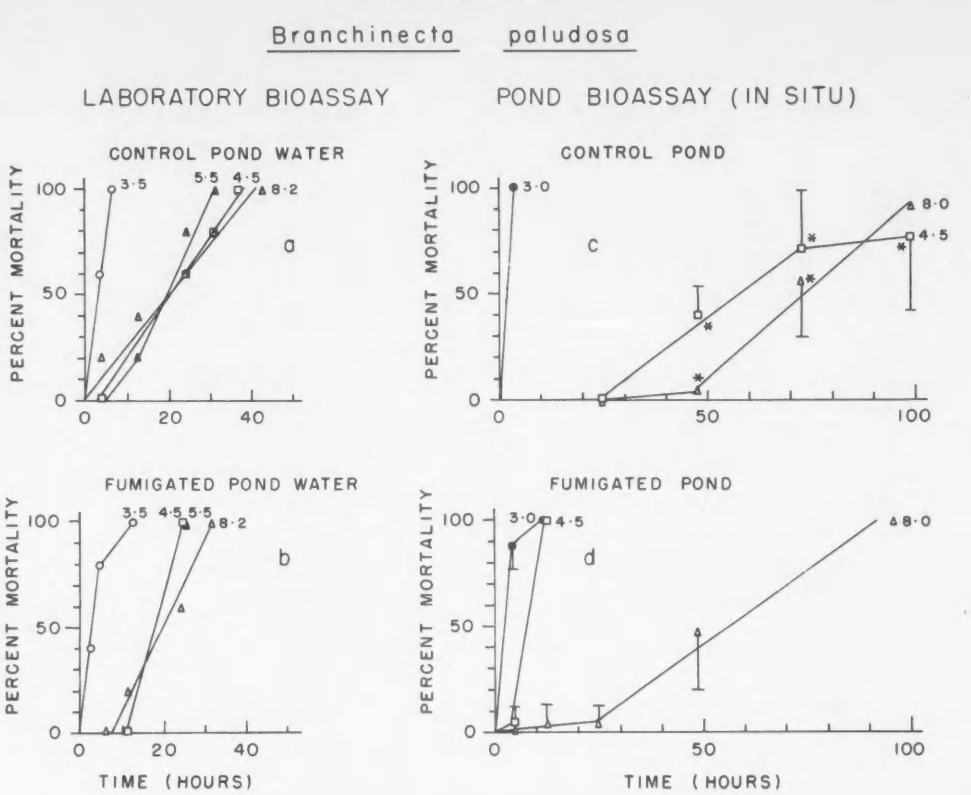


FIG. 3. Mortality curves for the fairy shrimp, *Branchinecta paludosa*, at different pHs in laboratory and pond bioassays. Numbers represent experimental pH. (*Predation by *Orthocladius*.) Vertical bars represent 1 SD.

calcium can prolong survival at low pH. However, the addition of calcium to the fumigated pond water had no significant ameliorative effect on survival (Fig. 7b), and mortality was significantly more rapid than in control water. This was probably because Ca concentration in the fumigated water was already quite high (50 mg/L) and thus differences in mortality were not due to Ca.

Sodium was also added to the fumigated water. In the present study, addition of Na initially suppressed mortality, which subsequently accelerated (Fig. 7c). Sodium had no long-term beneficial effect in reducing mortality. It thus seems improbable that the different Na concentrations in the two ponds could account for the difference in mortality observed at pH 4.5 as shown in Fig. 1 and 7a. This is contrary to some recent work with fish and crustaceans. It is known, for example, that fish tend to be eliminated first from dilute lakes (Schofield 1976). "Good fisheries" in lakes with a "high" salt content can occur as low as pH 4.7 to 5.0 (Leivestad et al. 1976). Experiments with trout eggs following 6 mo exposure at pH 4.9 showed that addition of NaCl reduced mortality from 70 to 17%. Furthemore, it has been shown that crustaceans experience problems with Na regulation at low pH (Potts and Fryer 1979; Havas 1980). The major difference, once again, is that the Na concentration in the fumigated pond is considerably higher than in most soft-water environments.

A key factor limiting plant growth on acid soils is the toxicity of aluminum and manganese, where their enhanced solubility causes concentrations sufficiently high to interfere with root growth (Magistad 1926; Rorison 1960). Acidic conditions near smelters have also caused mobilization of clay elements, such as aluminum, resulting in phytotoxicity (Whitby and Hutchinson 1974). Runoff from such areas add potentially toxic heavy metals to adjacent water bodies (Hutchinson et al. 1976). The high concentrations of the clay elements Al, Mn, and Fe, as well as of heavy metals such as Ni and Zn, in the acidic Smoking Hills pond water (Table 1) suggested a number of factors which could result in increased toxicity to the crustaceans compared to that of the alkaline pond water.

The removal of heavy metals and aluminum by co-precipitation significantly reduced the toxicity of acid pond water to the crustaceans (Fig. 7). It seems that the more rapid mortality at pH 4.5 in the fumigated water prior to co-precipitation is largely accounted for by the presence, at

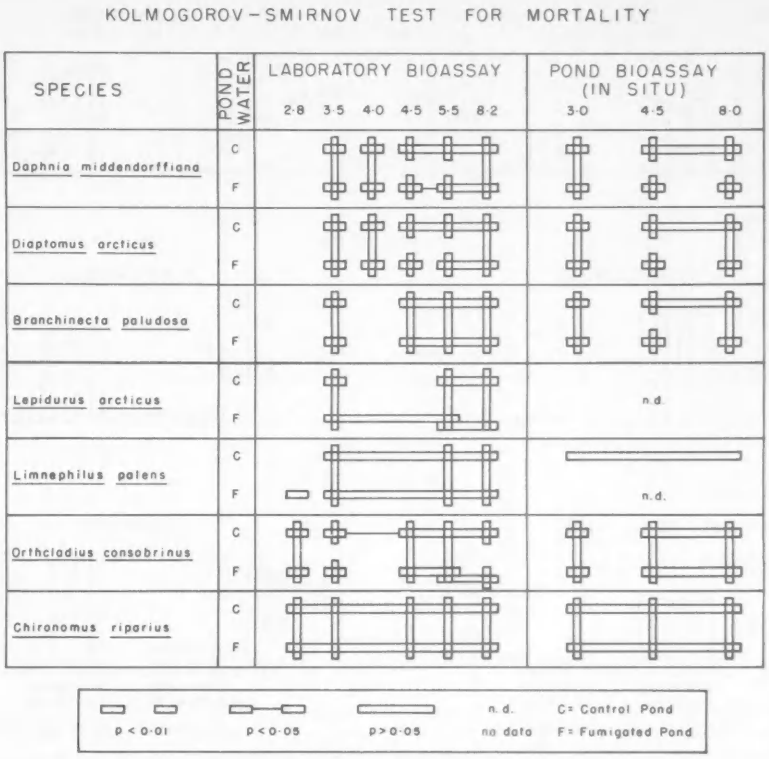


FIG. 4. Statistical analysis of mortality curves using Kolmogorov–Smirnov Test.

toxic concentrations, of one or more of these heavy metals. Figure 8 shows the results of adding to the control pond water, after readjustment to pH 4.5, the metals Ni, Zn, Fe, and Al on *D. middendorffiana* mortality.

Addition of 20 mg Al/L resulted in 100% mortality of *Daphnia* within 20 h (Fig. 8). The rate and degree of mortality now resembled that of fumigated pond water at pH 4.5 (Fig. 8a and e). Anderson (1948) has reported that ≤1.3 mg Al/L resulted in 50% immobilization of *D. magna* within 64 h. Biesinger and Christensen (1972) found that the 48-h LC50 was 3.9 mg Al/L for *D. magna*. In another experiment (Havas 1980), 1 mg Al/L added to the control water had no significant effect on *D. magna* mortality, while 5 mg Al/L resulted in rapid mortality (i.e. 50% mortality within 10 h). These reported lethal Al concentrations are considerably less than those occurring in the acid ponds at the Smoking Hills. The Al concentration alone, then, would be more than sufficient to account for the differences in survival time noted for *D. middendorffiana* between the two pond waters at pH 4.5, though at pH 4.5 some of the Al is precipitated.

The addition of 30 mg Fe^{+3}/L to control pond water resulted in rapid mortality of *D. middendorffiana* (Fig. 8d). However, this mortality cannot be attributed to the iron alone. Ferric ion (Fe^{+3}) acts as a buffer at low pH (Campbell et al. 1964). The addition of free Fe resulted in the formation of iron hydroxide and a drop in pH to 4.2 within 50 h. This lowered pH alone may be sufficient to account for much of the increased mortality, so that no unequivocal conclusion can be drawn from the Fe addition experiment. Anderson (1948) also reported changes in pH following Fe additions in his experiments. It seems probable, however, that the ferric ion exerted some toxicity at the 30-mg/L level. Iron may not have been a key factor resulting in mortality as the concentration at pH 4.5 was only 6 mg/L in the fumigated water (Fig. 6).

Nickel (at 0.20 mg/L) appeared to have no detrimental effect on mortality of *D. middendorffiana* when added to control water at pH 4.5. Indeed, mortality was significantly reduced by addition of Ni (Fig. 8b). Shcherban (1979) examined the effect of temperature on metal toxicity to *D magna* and found that even at 30°C there was 100% survival at 0.25 mg Ni/L. Anderson (1948) found that 0.32 mg Ni/L in Lake Erie water was the 64-h threshold concentration which resulted in 50% immobilization of *D. magna*. The 48-h LC_{50} has been reported to be 0.51 mg/L for *D. magna* (Biesinger and Christensen 1972) and 1.9 mg/L for *D. hyalina*. It is not surprising, therefore, that 0.2 mg/L of Ni added to the con-

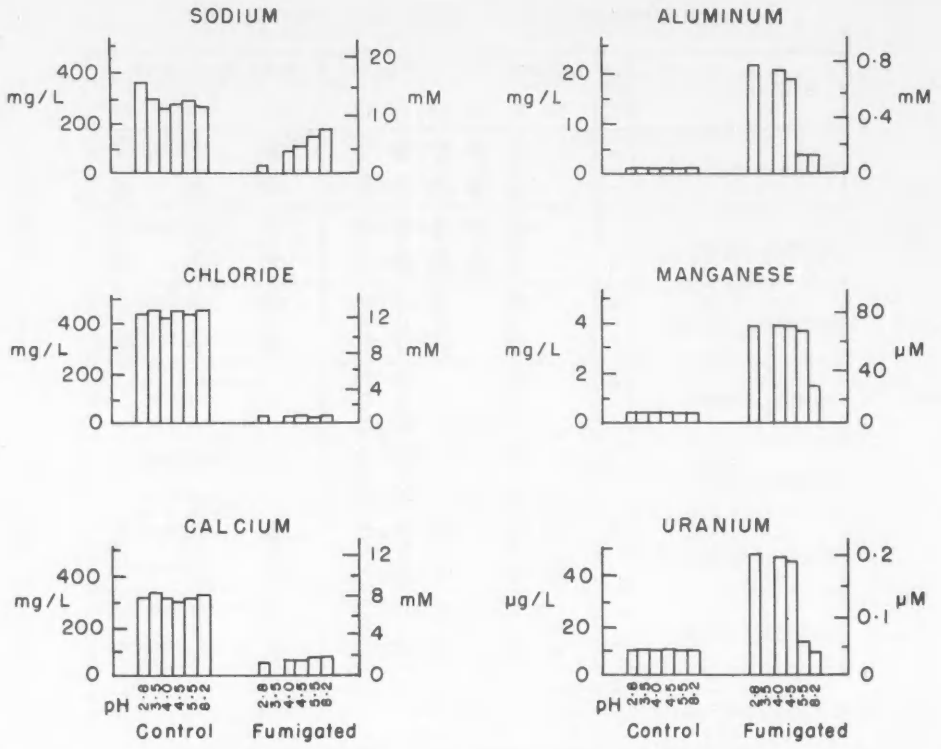


FIG. 5. Chemical composition of control and fumigated pond water at different experimental pHs in the laboratory bioassay, August 1976.

trol water did not account for the rapid mortality of *D. middendorffiana* in the fumigated water at pH 4.5.

The concentration of Zn found in the acid pond was 1.1 mg/L. Zinc appeared to have no detrimental effect on mortality of *D. middendorffiana* when added at this concentration to the control pond water (Fig. 8c). The 48-h LC50 for *D. magna* and *D. hyalina* was reported to be 0.1 and 0.04 mg Zn/L, respectively (Biesinger and Christensen 1972; Baudouin and Scoppa 1974), while Anderson (1948) found that 0.07 mg Zn/L resulted in 50% immobilization of *D. magna* within 64 h. The toxicity of the added Zn in the present study seems to be less than expected, especially because it has been shown that the toxicity of Zn increases at lower pH (Sprague 1975). The major difference between our experiments and those referred to above is that the Ca concentration in the control pond was very high (250 mg/L) and Ca has been shown to interact antagonistically with Zn (Hartung 1973; Sinley et al. 1974).

Addition of all four metals to the control pond water at concentrations occurring in the acid pond did not significantly increase *Daphnia* mortality over that of adding Al alone at 20 mg/L (Fig. 8f). Aluminum can, therefore, be considered a key additional factor to that of H^+ ion concentration per se in determining crustacean mortality in these experiments. Recent work with brook trout also identifies Al as a major toxin in acidified waters (Schofield 1977).

INSECT RESPONSES

The insect larvae were much more tolerant of low pH and were also more variable in their response than the crustaceans. While 50% mortality of crustaceans occurred within 12 h at pHs below 4.5, all three insect species tested could survive at pH 3.5 from 50 h to 20 d (Fig. 9–11). Individuals of the caddis fly, *Limnephilus pallens*, survived for 38 d, at which time the laboratory experiment was terminated (Fig. 9).

The red chironomid (*Chironomus riparius*), isolated from the fumigated pond, was not only able to survive much better in low pH waters than the other chironomid species tested, but also survived well in alkaline water (cf. Fig. 10 and 11). Neither pH nor pond water chemistry significantly affected the rate of mortality (Fig. 11). The sole exception was at pH 2.8 in the control pond water where initial mortality increased over that from pH 3.5 to 8.2 (Fig. 11a). In all experiments with *C. riparius*, death of individuals was preceded and could be accurately predicted by the loss of the red pigmentation (hemoglobin). This generally began 24 h prior to death.

Hemoglobin may be a critical factor in chironomid survival

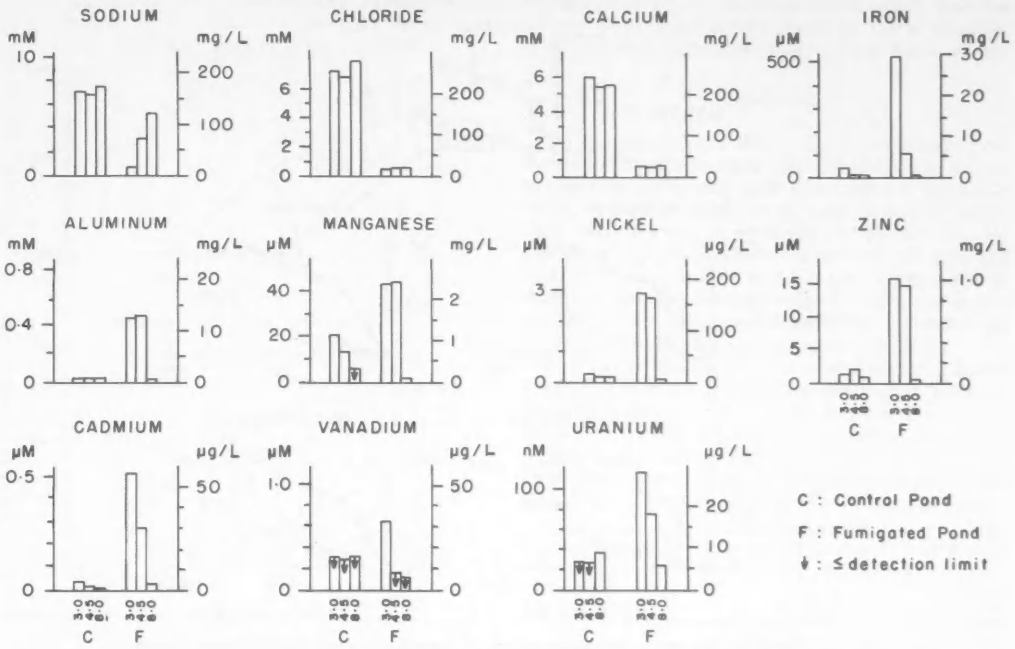


FIG. 6. Chemical composition of control and fumigated pond water at different experimental pHs, in the pond bioassays, July 1977.

at low pH. According to Jernelov et al. (1981), the hemoglobin acts as an internal buffer. The buffering capacity of the hemoglobin in the Smoking Hills red chironomids is greater than that of similar species collected in Sweden. The loss of pigmentation which occurs prior to mortality may be caused by denaturation of the hemoglobin molecule as a result of hydrogen ion penetration.

In slightly less acidic environments in Sweden and Norway (pH 3.9 to 4.6), while there is an overall decrease in the zoobenthos compared with less acidic lakes, chironomids dominate the community (Leivestad et al. 1976; Wiederholm and Erikkson 1977).

The other dipteran, *Orthocladius consobrinus*, lacked significant hemoglobin and was considerably more sensitive to low pH (Fig. 10). However,there appeared to be considerable variability within the population. A large percentage (90−95%) of larvae died rapidly (within 50 h) at pH 2.8 in control water and at pH 3.5 in the fumigated water (Fig. 10a and b). However, a few individuals were able to survive under these extremely acidic conditions for more than 300 h, at which point the experiment was terminated. This suggests that while most of these green chironomid larvae would be rapidly eliminated in acidic ponds, there is a possible genetic pool of diversity available for selection and evolution of more acid-tolerant populations. Such a mechanism of selection may have established the population of red chironomid larvae in the strongly acidic ponds. While *O. consobrinus* was abundant in the alkaline ponds, it was rare or absent from the acidic ponds at the Smoking Hills (Hutchinson et al. 1978).

Limnephilus pallens, the caddis fly larvae, were also relatively tolerant of low pH but were restricted to alkaline ponds at the Smoking Hills. Mortality of this species appeared to be more rapid in non-fumigated water than in fumigated water between pH 3.5 and 8.2 (Fig. 9). This was also found to be the case for *C. riparius* at pH 2.8 (Fig. 6). Though the reason for this is not clear, it is suspected that the increased salinity in the fumigated waters may be a factor enhancing survival. The caddis fly larvae was not found in any of the acid ponds we sampled ($n \geq 20$). Yet, in the bioassays it showed remarkable tolerance to extremely acidic conditions in both pond waters. Its absence from the acid ponds is intriguing and must relate to factors not assessed here. These include the possible absence of suitable food in the acid ponds, potential sensitivity during emergence in acid waters, and absence of suitable material for building its casing in the acid ponds. Submerged dwarf willow, which it uses for casing material at the Smoking Hills, was abundant only in the alkaline ponds.

It is recognized in evaluating these bioassays that survival of a particular stage in the life cycle under 'artificial conditions' does not determine field distribution. Organisms must be able to reproduce successfully to survive, and to compete successfully if they are to be found in a particular environment. Food sources can be critical. In these experiments only survival was tested.

Bell (1971) found that with respect to pH, emergence was the most critical stage for insects. Of the nine species of insects he tested, the pH required for 50% successful emergence was on average one pH unit greater than that

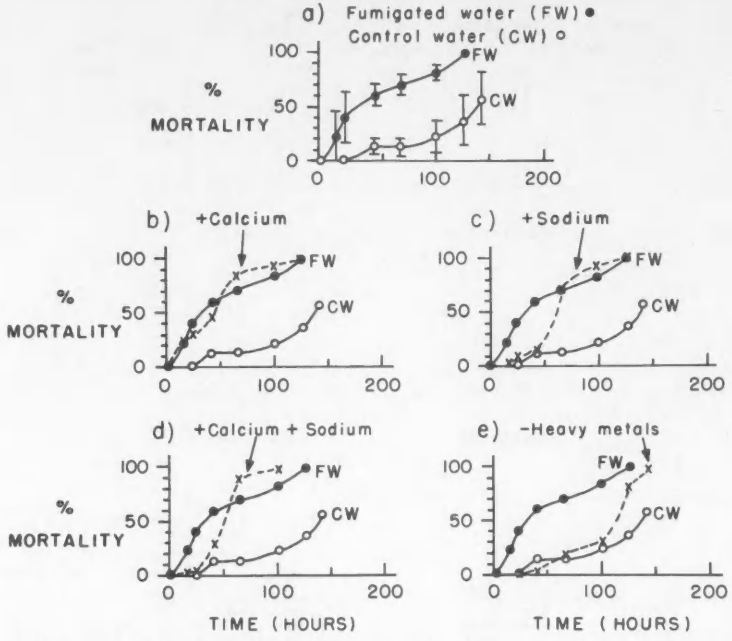


FIG. 7. Mortality of *Daphnia middendorffiana* at pH 4.5. Amendments were made to the fumigated pond water, 1978. Vertical bars = 1 SD.

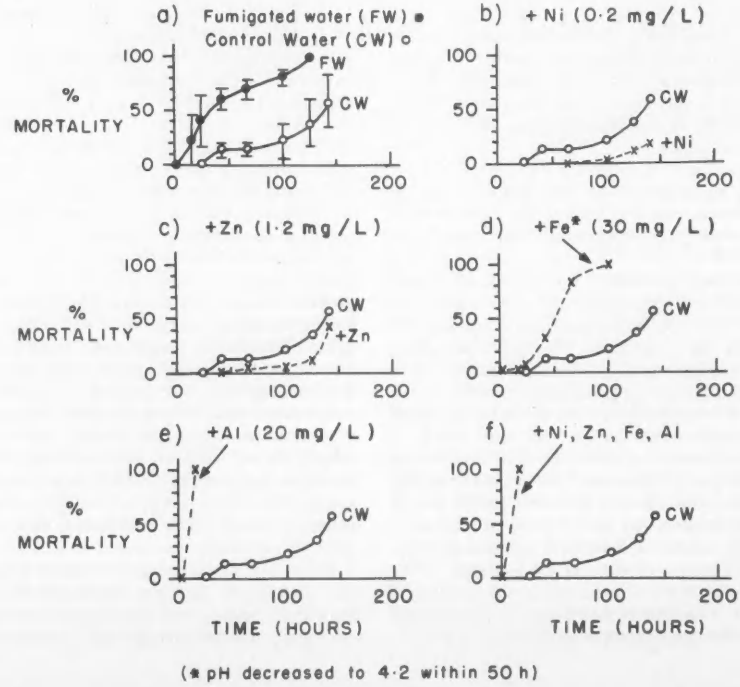
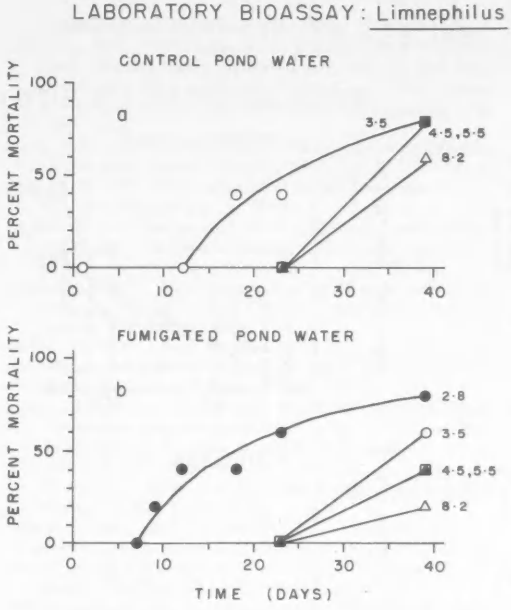


FIG. 8. Mortality of *Daphnia middendorffiana* at pH 4.5. Amendments were made to the control pond water, 1978. Vertical bars = 1 SD.



obtained for 30 d TL50. Davis and Ozburn (1969) found that although *Daphnia pulex* could survive for 32 h at pH as low as 4.2, successful reproduction did not occur below pH 7.0.

Conclusion

The experiments have shown that the crustaceans from Smoking Hills alkaline ponds were very sensitive to low pH and died rapidly in pond water below pH 4.5. The insect larvae, in contrast, whether from acidic or alkaline ponds, were able to survive in water down to pH 3.5. The red chironomid from the acid pond was not only able to survive at pH 2.8, but also in water as high as pH 8.2. The caddis fly larva from alkaline ponds, but which does not occur in acid ponds at Smoking Hills, survived for extended periods at pH 3.5 while several individuals also survived at pH 2.8.

To determine whether pH per se or additional toxic factors were responsible for the observed mortality in bioassays involving fumigated pond water, the importance of Ca, Na, Fe, Al, Zn, and Ni was considered. Experimental evidence strongly suggests that Al is the key additional toxic factor in the fumigated water. Ferric ion may also contribute to toxicity. Calcium additions did not alleviate this heavy metal

FIG. 9. Mortality curves for *Limnephilus pallens* (Trichoptera) at different pHs in the laboratory bioassay. Numbers represent experimental pH.

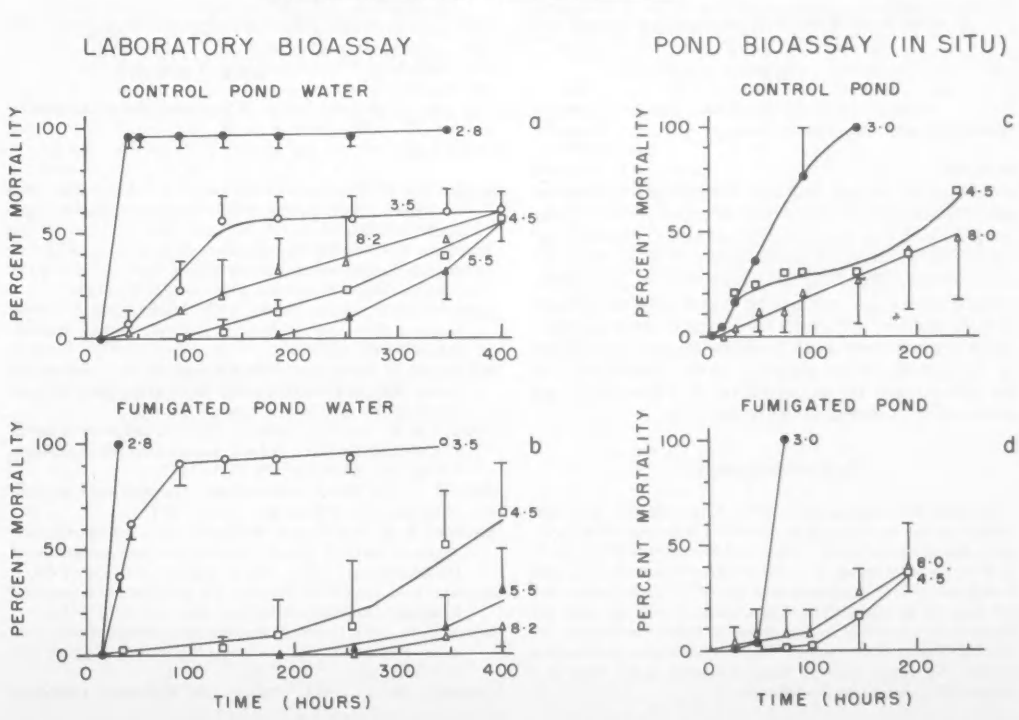


FIG. 10. Mortality curves for *Orthocladius consobrinus* (Diptera) at different pHs in laboratory and pond bioassays. Numbers represent experimental pH. Vertical bars = 1 SD.

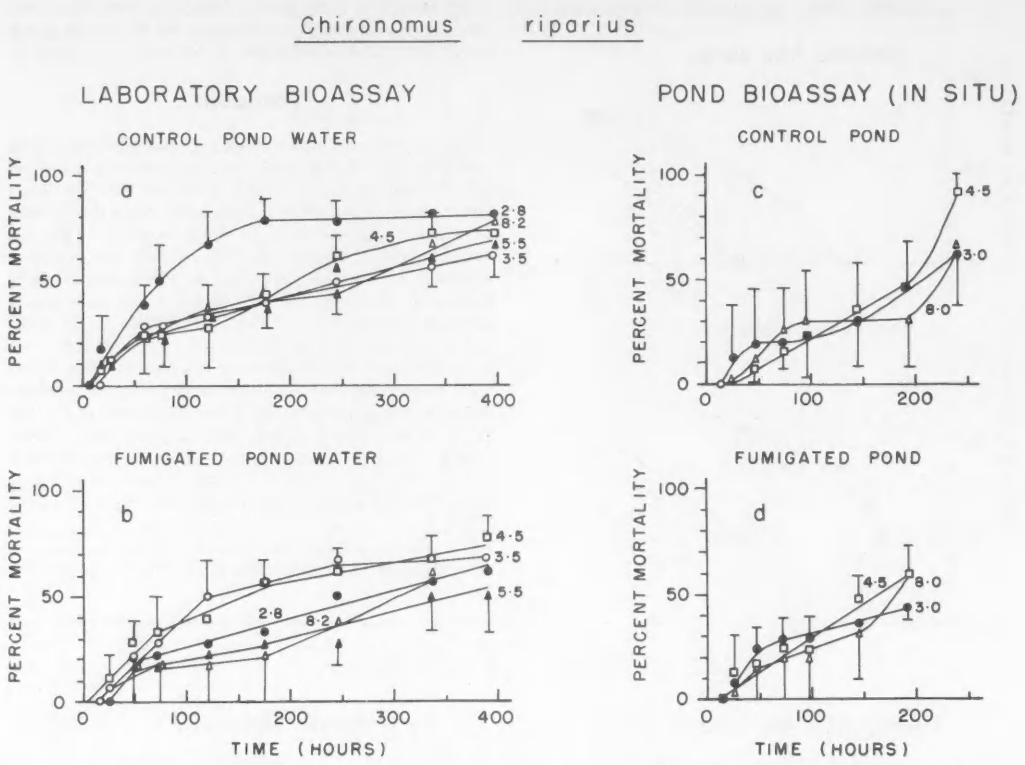


FIG. 11. Mortality curves for *Chironomus riparius* (Diptera) at different pHs in laboratory and pond bioassays. Numbers represent experimental pH. Vertical bars = 1 SD.

toxicity, while sodium had only a temporary ameliorative effect. While selection and physiological adaptation of alkaline pond biota may have played a role in the occurrence and survival of crustaceans in ponds adjusted to pH as low as 4.5 at the Smoking Hills, it appears that for the very acidic ponds, with pH down to 2.8, colonization by acid-tolerant organisms de novo may best account for the present differences in species between the most acidic ponds and the ones less affected by fumigations. Water chemistry alone is unlikely to be the direct cause of the restriction of *Orthocladius* and *Limnephilus* to alkaline waters at the Hills.

Acknowledgments

We thank Beth Hutchison for skilled field assistance. Invaluable logistic support was supplied by the Polar Continental Shelf Project of the Department of Energy, Mines and Resources at Tuktoyaktuk, N.W.T. Financial assistance for the Smoking Hills studies has come from grants to T. C. Hutchinson from the NATO Eco-Sciences panel, the National Research Council of Canada, the Arctic Land Use Research Programme of the Department of Indian and Northern Affairs, and from the Department of Fisheries and Marine, Environment Canada. M. Havas received financial support in the form of a National Research Council scholarship.

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Nonlinear Relation Between Smolts and Adults in Babine Lake Sockeye Salmon (*Oncorhynchus nerka*) and Implications for Other Salmon Populations

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PETERMAN, R. M. 1982. Nonlinear relation between smolts and adults in Babine Lake sockeye salmon (*Oncorhynchus nerka*) and implications for other salmon populations. Can. J. Fish. Aquat. Sci. 39: 904–913.

Data on sampling variability in smolt abundance for Babine Lake sockeye salmon (*Oncorhynchus nerka*) are combined with a previous analysis to calculate a more accurate estimate of the degree of nonlinearity in the relation between smolts and adults. Results indicate significant within-stock nonlinear mortality, large variability in mortality which tends to obscure any smolt-to-adult relation, or both. Analysis of age structure data identifies the first 15 mo of marine life as the period when most of this nonlinear or relation-masking mortality occurs. I also calculate the amount of smolt measurement error below which other salmon stocks are classed as having nonlinear marine survival. A distinct separation between even and odd brood year marine survival of Babine Lake sockeye suggests interactions with pink salmon. Juvenile pink salmon (*O. gorbuscha*) abundances correlate positively with residuals in Babine Lake sockeye survival for the same seaward migration year. This suggests a depensatory mortality effect which occurs later in the marine life stage than the possible within-population compensatory effect. Depensation is plausible because the size of pink fry equals that of sockeye smolts by August in coastal waters, permitting mutual swamping of predators. The paper concludes with implications for further enhancement.

Key words: density dependence, marine survival, measurement error, enhancement, depensation, nonlinear, variability

PETERMAN, R. M. 1982. Nonlinear relation between smolts and adults in Babine Lake sockeye salmon (*Oncorhynchus nerka*) and implications for other salmon populations. Can. J. Fish. Aquat. Sci. 39: 904–913.

Combinées à une analyse antérieure, les données sur la variabilité de l'échantillonnage de l'abondance des smolts de saumon nerka (*Oncorhynchus nerka*) du lac Babine ont permis d'estimer plus précisément le degré de non-linéarité de la relation entre smolts et adultes. Les résultats indiquent qu'il y a forte mortalité non linéaire parmi le stock, qu'une grande variabilité de la mortalité tend à obscurcir toute relation entre smolts et adultes, ou que les deux peuvent se produire. D'après l'analyse de la structure par âge, on constate que les 15 premiers mois de vie en mer sont ceux pendant lesquels se produit la plus grande partie de cette mortalité non linéaire ou la mortalité masquant la relation. Nous calculons également l'erreur de mesure d'abondance des saumons au-dessous de laquelle d'autres stocks de cette espèce sont considérés comme ayant une survie en mer non linéaire. Comme semble l'indiquer une séparation distincte entre la survie en mer des saumons nerka du lac Babine d'années impaires et paires, il y aurait interaction avec le saumon rose (*O. gorbuscha*). Il y a corrélation positive entre l'abondance des jeunes saumons roses et ce qui reste des survivants des saumons nerka du lac Babine pendant la même année de migration vers la mer. Ceci indiquerait un effet de mortalité anticompensatoire pendant la phase de vie en mer survenant plus tard que l'effet compensatoire possible au sein de la population. L'effet anticompensatoire est plausible parce que la taille des alevins de saumon rose est égale à celle des smolts de saumon nerka en août dans les eaux côtières, ce qui permet l'écrasement mutuel des prédateurs. Nous terminons par une discussion des implications d'une mise en valeur accrue.

Received September 10, 1981
Accepted February 4, 1982

Reçu le 10 septembre 1981
Accepté le 4 février 1982

Printed in Canada (J6627)
Imprimé au Canada (J6627)

AN important question for salmon managers and researchers is whether adult abundance of a salmon stock increases significantly with increased abundance of juveniles entering the ocean. This positive association is assumed valid by designers of the numerous salmonid enhancement projects that are presently in operation in North Pacific Rim countries. My purpose here is to test this assumption for one particularly well-studied salmon stock and to relate the results to other salmon populations.

In a previous paper (Peterman 1978), I examined data on eight different stocks of Pacific salmon for evidence of density-dependent marine survival. The following relation was used to express survival through the marine life stage:

$$(1) \quad A = cS^k \exp(v)$$

where A = abundance of returning adults, S = abundance of smolts (or fry in the case of pinks and chums), v = normally distributed random variate with mean zero and variance σ^2, and c, k = constants.

Analysis of marine survival data shows that the multiplicative log-normal random noise term used in equation (1) is more consistent with the data than a normally distributed additive term (Peterman 1981). When k in the above equation is less than 1.0, there is a nonlinear relation between smolts and adults, and an increase in smolt abundance may not consistently lead to significantly more adult returns.

In the 1978 paper, I did not obtain a reliable estimate of the degree of nonlinearity in the smolt-to-adult relations because the statistical methods are sensitive to the magnitude of measurement, or observation, errors in smolt abundances. Estimates of these observation errors were not available at the time of that work, so the two statistical approaches which were used merely bracketed the degree of nonlinearity. As discussed in Peterman (1978), the geometric mean functional regression method (Ricker 1973) produces conservative estimates of the degree of nonlinearity, whereas the predictive regression, a special case of the Morris (1959) method, produces overestimates.

Kuno (1971) derived a method which adjusts the estimate of nonlinearity for measurement error in the X variate if the magnitude of the error is known. As well, MacDonald and Smith (1980) recently published a procedure which was used to calculate standard errors in estimates of annual smolt abundance for Babine Lake sockeye salmon (*Oncorhynchus nerka*), in British Columbia. In this paper, I use these standard error data along with Kuno's method, to correct the earlier estimate of the degree of nonlinearity in the predictive relation between smolts and resulting adults of the Babine stock. As I show later, adult returns vary considerably for a given smolt abundance in this stock. This observation supports the idea that several important factors may be missing from the predictive relation of equation 1. However, fitting this relation does permit calculation of residuals to test hypotheses concerning the source of that variability.

Methods

DATA SOURCES

Babine Lake sockeye migrate along the Skeena River, which is in Fisheries Statistical Area 4 in British Columbia. These salmon are mainly sub 2's, that is, they are in freshwater for almost 2 yr before migrating to sea as smolts (Larkin and MacDonald 1968). For example, offspring from the 1970 spawning migrated seaward in 1972. Because adults of this stock mainly return to their home river at ages 4 and 5, adult data are tabulated by age and year in which they were spawned (brood year). The Babine Lake sockeye data used in Peterman (1978) and Ricker and Smith (1975) are supplemented with smolt size and adult abundance data for brood years 1973–76 (A. D. Anderson and R. Kadowaki, Canadian Department of Fisheries and Oceans, Prince Rupert, B.C., personal communication). Peterman (1978) describes the sources of adult abundance data.

Each year's smolt body size figure is a weighted average of the early and late Babine Lake smolt runs. Smolt abundances are estimated at the Babine Lake counting fence, as are returning jacks (age 3). Jack abundance includes the catch of jacks in the dip-net fishery at the fence as well as escapement. Because jacks are rarely caught by commercial gear, this represents a complete estimate of age 3 returns. Abundance data are in millions of fish. H. Smith (Canadian Department of Fisheries and Oceans, Vancouver, B.C., personal communication) provided estimates of annual smolt numbers and their standard errors for brood years 1959–76, as estimated by the "parsimonious model" method in MacDonald and Smith (1980). When comparing results here with those in Peterman (1978), readers should note that annual smolt abundance estimates derived from MacDonald and Smith's method differ from those used in my 1978 paper, but the data sets are well correlated ($r = 0.965$). R. Gunsolus (Oregon Dep. of Fish and Wildlife, Clackamus, OR, personal communication) provided two more recent years of smolt-to-adult and jack data for Oregon Production Index area coho salmon (*O. kisutch*) to update information in Gunsolus (1978). Complete data for smolt release years 1960–79 were analyzed.

CORRECTION FOR MEASUREMENT ERROR

As is well known, the slope of a regression of Y on X is biased below its true value if X has a measurement error. Kuno's formula for the relation between the estimated slope (b') and the true slope (b) is:

$$(2) \quad b' = \frac{b}{1 + \lambda},$$

where λ is related to the magnitude of the measurement error in X. In the salmon case described by equation (1), the parameter k, equivalent to b' above, is estimated by the slope of the predictive regression of $\log_e A$ on $\log_e S$ (see Peterman 1978). This is the Morris method of estimating density dependence, or more specifically here, nonlinearity. Thus, we are concerned with what Kuno (1971) refers to as his test A, which deals with data in successive life stages in different years. The true degree of nonlinearity, b, is then estimated by rearrangement of equation (2):

$$(3) \quad b = b'(1 + \lambda)$$

Kuno defines λ as

$$(4) \quad \lambda = 0.1886\,\overline{D^2}/[s^2(\log S) - 0.1886\,\overline{D^2}]$$

where s^2 (log S) is sample variance for observed values of log

S. $\overline{D^2}$ is the mean of all values of D^2 for all annual samples, and D represents sample standard error per sample mean. D and D^2 values are thus calculated for each year. The constant in equation (4) arises from using logarithms.

In this paper, I clearly distinguish two components of a test for density-dependent marine survival. One is a test for nonlinearity for which the null hypothesis is $k = 1$ in equation 1. Here I use a one-tailed t-test to determine if the corrected slope of k, estimated from equation (3), is significantly <1.0. If so, a nonlinear smolt-to-adult relation is demonstrated and attempts to increase smolt abundance may not have as large an effect on adult returns as expected. Such nonlinearity also means percent survival rate varies with smolt abundance. The second component is the test for a significant correlation in the log−log relation used to fit the parameter k above. Here, the null hypothesis is $r = 0$. The failure to reject the hypothesis $r = 0$ means that large variability in survival tends to obscure any simple relation between smolts and adults, hence the cause of this situation is called here relation-masking or relation-obscuring mortality. Density dependence refers to the case where both null hypotheses are rejected, i.e. there is significant nonlinearity in the untransformed data, and the transformed data show significant covariation.

Allowable Measurement Error for Other Stocks

Data on measurement errors in smolt abundances are not available for the Bristol Bay sockeye populations that showed partial evidence of nonlinearity (with the Morris test) in Peterman's (1978) analysis. However, we can use a table of critical values of Student's t-distribution and equations (3) and (4) to solve for the value of $\bar{D}$ (sample error per sample mean) below which we would have to accept the conclusion that these populations exhibit curvilinearity between smolt and adult returns. The following procedure is used:

1) The critical t-statistic is found for $P = 0.05$ and the appropriate sample size. As we are concerned with cases where $b < 1.0$, we use a negative critical t value and a one-tailed test. Because the test for slope less than 1.0 is (Sokal and Rohlf 1969):

$$t = \frac{b - 1.0}{s_b},$$

we can solve for the value of b which is <1.0 with $P = 0.05$. The standard error of b' is justifiably used here because Ricker (1973) shows it to be applicable to the functional regression as well as to the predictive regression coefficients.

2) From a rearrangement of equation (3)

$$\lambda = \frac{b}{b'} - 1.0.$$

3) Equation (4) is rearranged and solved for $\overline{D^2}$ by substituting values of λ from above and s^2 (log S) from Table 2.

4) Taking $\sqrt{\overline{D^2}}$ gives the average sample error per sample mean ratio above which we cannot reject the null hypothesis that $b = 1.0$.

Critical Period

While the parameter k of equation (1) estimates the degree of nonlinearity in the total smolt-to-adult period, it does not identify the period within that life stage when most of the nonlinear processes operate or when variability in the mortality processes tends to obscure the relation between smolt abundances and adult returns. I therefore perform another type of analysis using data on total returns (catch plus escapement) of different-aged Babine Lake sockeye from the same brood year. If, for example, returns of age 4's in year t are linearly related to returns of age 3's in year $t - 1$ over a number of years, then I conclude that survival from age 3 to age 4 is not affected by abundance of that stock. Therefore, another period in marine life largely encompasses the nonlinear processes or those processes acting to mask the smolt-to-adult relation. Such a linear relation between 3's and 4's also means that the proportion of the ocean population of age 4's that migrates back to home waters is not related to salmon density (Peterman unpublished data).

Peterman (unpublished data) describes in detail the methodology for testing for linearity between ages, and it closely parallels the methods applied to equation (1) above by Peterman (1978). I use equations which relate abundances of successive ages for returns of a given brood class. A parameter d represents the degree of nonlinearity between abundances of successive ages, and it is compared to the null hypothesis, $d = 1.0$, by a two-tailed t-test. A standard predictive regression is used for the relation between ages 4 and 3 because 3's are accurately estimated at the Babine Lake counting fence. For comparison, the functional regression result is also shown. A functional regression is used for the relation between 5's and 4's because the latter data may include significant measurement error from catches or escapements. The parameter d is estimated from regressions in which both variables are transformed by taking natural logarithms to normalize the noise terms.

Even/Odd-Year Survival

Pink interaction — There is a large difference in marine survival of Babine Lake sockeye between even and odd brood years and I first hypothesize an interaction with pink salmon (*O. gorbuscha*) fry or returning adults. As described in Peterman (1981), deviations in Babine Lake sockeye marine survival are calculated by rearrangement of equation (1):

$$(5) \quad v = \log_e(A/cS^k).$$

These brood year residuals in survival are then correlated with pink salmon abundance in two ways. First, estimated juvenile pink abundances are paired with survival residuals of the sockeye smolt run of the same year (progeny of the brood 2 yr earlier). Second, adult pink salmon returns of a given year are paired with survival residuals of sockeye smolts going to sea that year. The former tests for common effects among juveniles of the two species, and the latter for interactions between adult pinks and sockeye smolts.

Annual adult pink salmon returns for all statistical areas in B.C. are derived from catches and escapements. Catch data are reported in the British Columbia Catch Statistics, published by the Canadian Department of Fisheries and Oceans, Vancouver, and escapements are reported in stream catalogues, now being produced in a series of Data Reports for each statistical area by the same organization. Escapement data for areas not yet covered by published catalogues are

TABLE 1. Rounded observation error statistics for Babine Lake sockeye smolts.

Smolt migration year (brood year +2)	D (sample SE/ sample mean no. of smolts)	D^2
1961	0.077	0.0059
1962	0.196	0.0383
1963	0.051	0.0026
1964	0.138	0.0192
1965	0.060	0.0035
1966	0.043	0.0019
1967	0.042	0.0018
1968	0.072	0.0052
1969	0.070	0.0049
1970	0.065	0.0042
1971	0.025	0.0006
1972	0.039	0.0015
1973	0.041	0.0017
1974	0.028	0.0008
1975	0.030	0.0009
1976	0.082	0.0068
1977	0.039	0.0015
1978	0.075	0.0056
s^2 (log S) = 0.064		$\overline{D^2}$ = 0.0059

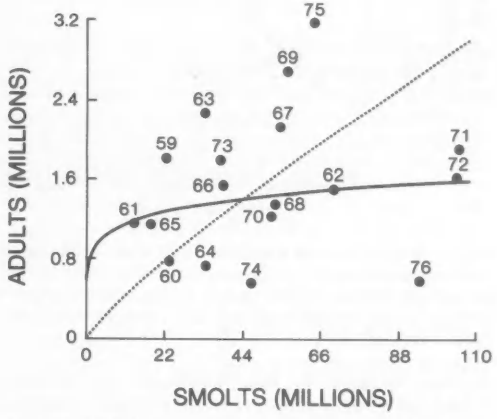

FIG. 1. Relationship between adult returns and smolt abundance, by brood year, for Babine Lake sockeye salmon; these smolts enter the ocean 2 yr after being spawned. Broken line is the functional regression estimate and solid line is the more correct relation which takes into account data on smolt observation errors. Solid line equation is $A = 0.799\ S^{0.149}$ where A and S are in millions of fish.

from Fisheries Officers' records (through F. Wong, University of British Columbia, Vancouver). I used a process analogous to cohort analysis (Pope 1972) to estimate crudely juvenile pink salmon abundances. I shifted annual pink adult returns backwards 1 yr to estimate the minimum number of pinks that must have entered the ocean to produce that many adults the following year. Because the values of marine survival rates of pink salmon are unknown for most statistical

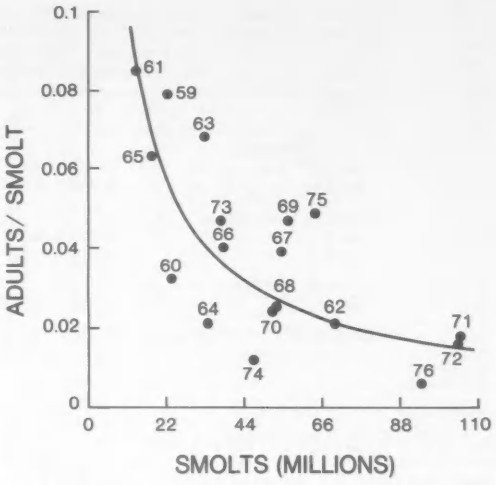

FIG. 2. Adults per smolt as a function of smolt abundance, by brood year, for Babine Lake sockeye. Curve is from equation (6) with the same c and k values as the solid line in Fig. 1.

areas, I did not divide by any such "blowup" factors to inflate adult numbers back to juveniles. If quite different and incorrect survival rates had been used for these areas, interpretation of results would have been confounded. Therefore, I assumed a constant, zero mortality rate for all areas, which yields a minimum estimate of juvenile pink abundance, giving equal weight to all areas considered. Pink salmon data were treated in two blocks: all statistical areas combined, and areas 1–10 combined. The latter block represents central and northern British Columbia stocks.

Smolt weight — I also used Bilton's (1971) hypothesis concerning the relation between smolt size and mean age of maturity to attempt to explain the difference between even and odd brood year survival for Babine Lake sockeye. While Bilton's hypothesis predicts alternation in mean age of return between successive generations, some of his relations could possibly affect age of maturity and marine survival in alternate years. The proportion of adults returning at age 4 is related to smolt weight for each brood year. Smolt weights and residuals in weights from their parabolic time trend are compared for even and odd years. Marine survival residuals are related to proportion of adult sockeye returning as age 4, and a comparison is made of the proportion of age 4 adults in successive generations.

Results

CORRECTION FOR MEASUREMENT ERROR

Table 1 gives some intermediate data in the calculation of λ for Babine Lake sockeye, and λ is found to be 0.0177 from equation 4. Because $b' = 0.146$, as estimated by the log–log regression of adults on smolts, the best estimate for k from b in equation (3) is 0.146 (1 + 0.0177) = 0.149 for brood

TABLE 2. Rounded statistics for calculating the maximum variability in smolt abundance estimates that would still permit the conclusion that a nonlinear relation between smolts and adults occurs in Oregon coho and several Bristol Bay sockeye salmon populations. Symbols are defined in text except for $\bar{D}_{Bab.}$ which is the average standard error to mean ratio for abundance of Babine Lake sockeye smolts.

Stock	n	df	b'	$s_{b'}$	$t_{0.05\ (df.)}$	s^2 (log S)	λ	$\bar{D}$	$\bar{D}/\bar{D}_{Bab.}$
Kvichak R. sockeye									
sub 2's	19	17	0.708	0.128	−1.740	0.604	0.098	0.533	8.18
sub 3's	19	17	0.706	0.099	−1.740	0.737	0.173	0.759	11.65
Naknek R. sockeye									
sub 2's	17	15	0.290	0.205	−1.753	0.148	1.211	0.656	10.07
sub 3's	14	12	0.512	0.177	−1.782	0.092	0.337	0.351	5.39
Ugashik R. sockeye									
sub 3's	14	12	0.702	0.115	−1.782	0.225	0.132	0.373	5.73
Oregon coho	20	18	0.504	0.218	−1.734	0.029	0.233	0.171	2.63

years 1959−76. The standard error of this corrected value of k is unknown, so using the standard error (=0.213) of the uncorrected k value, the corrected k is found to be significantly less than 1.0 by means of a t-test ($P < 0.005$).

Figure 1 shows a plot of the 1959−76 brood year data with the functional regression estimate of the relation and the estimate which is corrected for smolt measurement error. From the above results I conclude that adult returns of Babine Lake sockeye are best predicted by a nonlinear relation with smolt abundance. This implies that survival rate is also nonlinear with respect to smolt abundance. However, the lack of significant correlation between natural logarithms of smolts and adults ($r = 0.17$, $P_{r=0} > 0.5$) means (1) that the above relation does not explain much of the variability, and (2) that the mortality agents operating in this life stage are so variable that they tend to obscure any relation between these two variables, i.e. adults vary around some constant average value. When one interprets either the result of nonlinearity or lack of correlation, the conclusion is the same; increases in number of smolts do not result in significant increases in average adult returns for Babine Lake sockeye.

Figure 2 shows the marine survival rate (adults per smolt) as a function of smolt abundance, by brood year. The curve is drawn from a rearrangement of equation (1):

$$(6)\quad A/S = cS^{k-1}$$

with the same c and corrected k values as reflected by the solid line in Fig. 1. This avoids the problem of interpreting the statistics of a curve fit to the data shown in Fig. 2, where the X variate is contained in the Y.

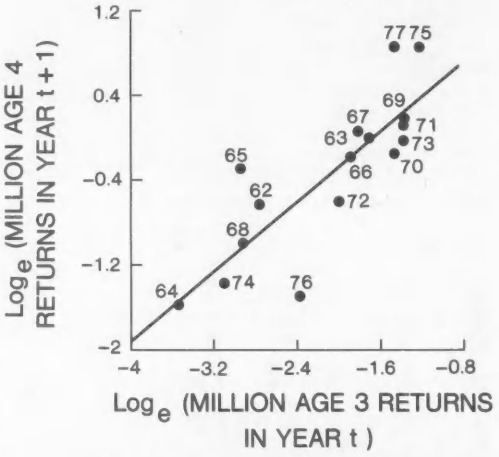

FIG. 3. Relation between abundances of age 3 returns of Babine Lake sockeye salmon in a given year and age 4 returns in the following year. Abundances are expressed as millions of fish and brood years are denoted beside points. The equation is $\log_e$ (age 4) = 1.361 + 0.819 $\log_e$ (age 3); $r = 0.83$ and $P_{r=0} < 0.001$.

ALLOWABLE MEASUREMENT ERROR FOR OTHER STOCKS

For the Babine sockeye, observation errors are relatively small and so the best estimate of the parameter k is fairly close to the original estimate b' from the predictive regression of $\log_e A$ on $\log_e S$. If other salmon stocks have similar small magnitudes of observation errors, then nonlinearity may be shown in the smolt-to-adult periods of many of the remaining 5 out of 10 cases that were classed in Peterman (1978) as density dependent by only the liberal Morris test. The second-to-last column of Table 2 gives results of calculations of the critical ratio of sample standard error to sample mean for abundance of smolts for these five populations. For comparison, $\bar{D}$ for Babine sockeye is ~0.065. If true $\bar{D}$ values are greater than the critical ones shown in Table 2, then we cannot reject at the 0.05 level, the null hypothesis that marine survival varies randomly around a constant, which would give a linear smolt-to-adult relation ($b = 1.0$). Alternatively, if in reality $\bar{D}$ values are less than those shown, and if in reality $b = 1.0$, then there is a less than 5% chance of observing such a small slope. In the latter case we would conclude that marine survival is a nonlinear function of smolt abundance.

The last column of Table 2 takes the ratio of $\bar{D}$ for each stock to $\bar{D}$ for Babine sockeye. This ratio indicates how variable smolt sampling would have to be, relative to that at the Babine Lake counting fence, to conclude that these populations do not show nonlinearity in marine survival. For example, the standard error per mean ratio for Kvichak sub 2 smolt estimates would have to be more than 8.18 times as high

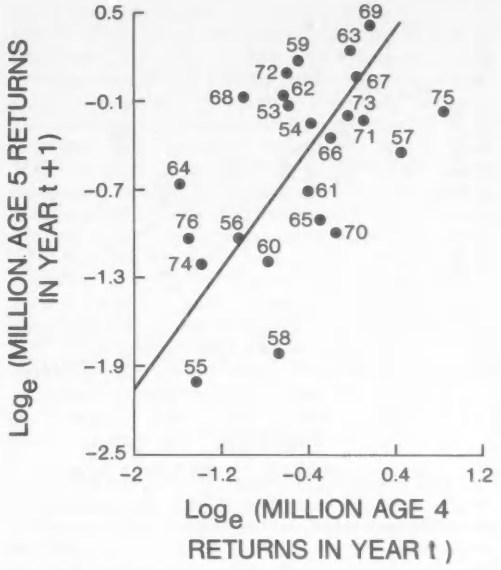

FIG. 4. Same as Fig. 3 except ages 4 and 5 are used. Log_e (age 5) = −0.029 + 1.021 log_e (age 4); $r = 0.54$ and $P_{r=0} < 0.01$.

as that ratio for Babine sockeye to conclude that $b = 1.0$ with $P > 0.05$, and that Kvichak sub 2 fish do not show nonlinearity in marine survival. If that ratio is less than 8.18, then we can conclude that the population likely does experience nonlinearity in survival. Of course, there could be a nonlinear effect which is masked by variability in other factors, even when this test does not demonstrate nonlinearity.

Bristol Bay sockeye smolt abundances for the years discussed here were mainly estimated by fyke net sampling (Paulus and Parker 1974). Unfortunately, there is no quantitative information to estimate $\bar{D}$ for these populations in those years. However, it is felt that the variability in smolt abundance estimates was large and probably greater than at least the low values of $\bar{D}$ in Table 2 (C. Meacham, Alaska Department of Fish and Game, Anchorage, AK, personal communication). Thus, the evidence on Bristol Bay sockeye is still inconclusive. After several more years of brood year adult returns, the analysis can be redone for post-1970 broods, for which more accurate sonar methods for smolt enumeration are available.

The Oregon coho situation falls into a category similar to the Bristol Bay sockeye. That is, the functional regression estimate of the degree of nonlinearity, k in equation (1), is not significantly different from 1.0, but the predictive regression (Morris method) estimate is ($k = 0.504$, $n = 20$, $P < 0.025$). If, according to Table 2, coho smolt abundances are relatively accurately measured with $\bar{D} < 2.63$ times that of Babine Lake sockeye salmon, then the true value of k is probably significantly less than 1.0. Because most Oregon Production Index coho salmon are of hatchery origin (Gunsolus 1978), their abundance probably is relatively accurately measured, but no good information is yet available to substantiate this presumption.

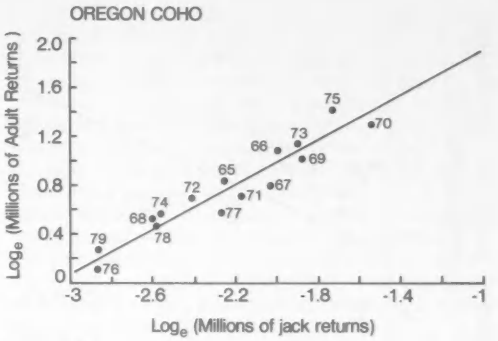

FIG. 5. Relation between abundances of jacks (age 2 returns) of Oregon Production Index area coho salmon in a given year and adult (age 3) returns in the following year. Abundances are expressed as millions of fish, and ocean entry years for coho smolts are shown. Log_e (adults) = 2.825 + 0.916 log_e (jacks); $r = 0.96$ and $P_{r=0} << 0.001$.

CRITICAL EARLY PERIOD

Nonlinear mortality or mortality which tends to obscure any relation between smolts and adults occurs in the Babine Lake sockeye population (Fig. 1 and 2) between the time when smolts are enumerated at the counting fence and the time when all brood year adults have returned (3 yr later). However, it is possible to identify more precisely the time period in which most of this nonlinear or relation-masking mortality occurs by determining if abundances of different-aged returns from the same brood are linearly related. Results are shown in Fig. 3 and 4 for ages 3−4 and 4−5. Neither age interval yields a d value significantly different from 1.0 (3-to-4 predictive regression: $d = 0.82 \pm 0.15$, $P > 0.1$; 3-to-4 functional regression: $d = 0.99 \pm 0.15$, $P > 0.5$; 4-to-5: $d = 1.02 \pm 0.18$, $P > 0.5$), and so these abundances are linearly related. Correlation coefficients are significantly different from zero for both ages; 3-to-4: $r = 0.83$, $P < 0.001$, 4-to-5: $r = 0.54$, $P < 0.01$. Therefore, most of the nonlinear or relation-masking mortality in the total smolt-to-adult life span must occur between the time when smolts are enumerated going downstream at the Babine fence (averaging late May) and when returning jacks from that same brood are counted at the fence in late August of the following year. This critical 15-mo period includes a period of freshwater downstream migration (~2 wk) and a short residence in the Skeena River estuary (up to 1 mo, Manzer 1956; Higgins and Schouwenburg 1973).

These results are similar to those described by Gunsolus (1978) for Oregon coho salmon. He also finds a linear relation between jacks and subsequent brood year adult returns, and he concludes that if density-dependent marine mortality affects that population, it must occur in the 4-mo period between smolt migration and September of the same year when jacks return. Figure 5 incorporates data from Gunsolus (1978, fig. 8) as well as additional data, and with the functional regression method I find $d = 0.92 \pm 0.075$,

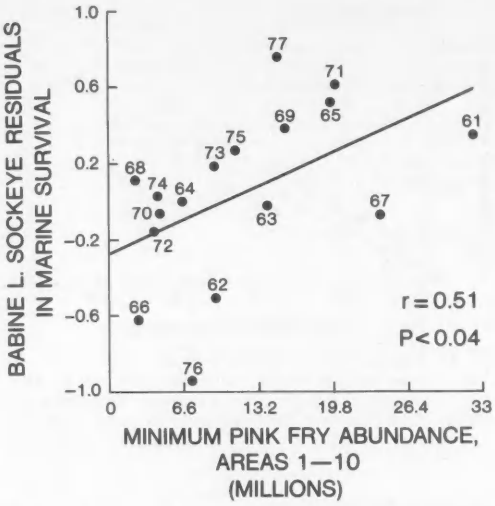

FIG. 6. Babine Lake sockeye marine survival residuals (calculated from equation 5) related to minimum estimated pink fry abundance in B.C. statistical areas 1−10 which migrate seaward in the same year as the sockeye smolts. By ocean entry year. $Y = -0.269 + 0.027X$; $r = 0.51$ and $P_{r=0} < 0.04$.

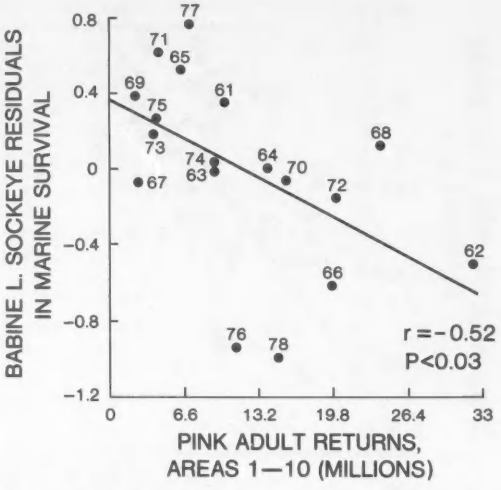

FIG. 7. Babine Lake sockeye marine survival residuals related to adult pink returns to areas 1−10 in the year of smolt migration. By sockeye ocean entry year. $Y = 0.362 - 0.031X$; $r = -0.52$ and $P_{r=0} < 0.03$.

which is not significantly different from $d = 1.0$ ($P > 0.1$). Therefore, Gunsolus' conclusions regarding linearity in the jack-to-adult period are substantiated.

Similar analyses of Bristol Bay sockeye salmon age structure data also confirm that mortality and proportion of the ocean population maturing at a given age are not related to abundance of each stock, except for Naknek River sockeye (Peterman unpublished data). Thus, as discussed earlier, if the smolt measurement errors for those populations are small enough for us to conclude that nonlinear or relation-obscuring mortality occurs somewhere in the smolt-to-adult life stage, then it most likely occurs in roughly the 1st year of smolt life.

The relations shown in Fig. 3−5 are useful to fisheries managers because forecasts of the following year's returns can be made from a given year's returns of young fish of the same brood class.

EVEN/ODD-YEAR SURVIVAL

Pink interaction — As illustrated for Babine Lake sockeye salmon in Fig. 1, a large component of the variance in survival to adults is associated with brood year; odd sockeye brood years tend to have greater adult returns than even brood years, for any given smolt abundance. The residuals in marine survival, as calculated by equation (5), are significantly and positively correlated with minimum estimated abundance of areas 1−10 pink salmon entering the ocean in the same year as sockeye smolts ($r = 0.51$, $n = 17$, $P < 0.04$), (Fig. 6). Survival residuals are also significantly correlated with adult pink returns for areas 1−10 in the year of smolt migration ($r = -0.52$, $n = 18$, $P < 0.03$) (Fig. 7), but are not correlated with either set of total British Columbia pink salmon

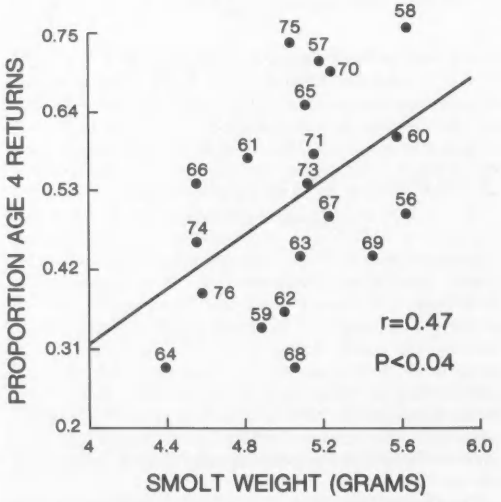

FIG. 8. Proportion of brood year adult returns migrating back at age 4 as a function of average smolt weight for that brood year, for Babine Lake sockeye, by brood year. $Y = -0.439 + 0.189X$; $r = 0.47$ and $P_{r=0} < 0.04$.

data, or area 4 pink data.

Smolt weight — An even/odd-year difference in Babine sockeye marine survival could conceivably also be explained by results which test Bilton's hypothesis that larger smolts (which reflect faster growth rates) tend to mature at an earlier age, as measured here by the proportion of 4's in each brood

year's adult production ($r = 0.47$, $n = 20$, $P_{r=0} < 0.04$) (Fig. 8). These results are virtually unchanged by using the arcsin transform of the proportion of 4's because extreme values do not appear in the data. Some Fraser River sockeye populations, for which little smolt size information is available, also mature earlier when marine growth is relatively fast (International Pacific Salmon Fisheries Commission 1962). However, there is no difference in the Babine sockeye between even and odd brood years' smolt weights or residuals in smolt weights from their time trend and no relation between the proportion of age 4's and brood year marine survival ($P > 0.5$). Smolt weight is also not related to smolt or fry abundance ($P > 0.05$) so the even/odd difference in Babine sockeye marine survival is not due to an interaction between smolt body size and age at maturity. There is also no alternation in the proportion of 4's between successive generations, as demonstrated earlier in Larkin and MacDonald (1968) and confirmed here with more recent data.

Discussion

These results show that adult returns of Babine Lake sockeye salmon are not significantly increased by increased smolt abundance. This could be due to (a) a nonlinear relation between smolts and their mortality rate during downstream migration or in marine waters, (b) large variability in mortality processes such that any underlying relation between smolts and adults tends to be obscured, or (c) a combination of (a) and (b). Ricker's (1976) review of ocean mortality suggests that the greatest magnitude of mortality occurs during early ocean life. Data discussed here show that most of the nonlinear or relation-masking mortality of the Babine Lake sockeye also occurs in this early period, specifically the first 15 mo.

Because little new can be said about sources of highly variable mortality, discussion here focusses on the potential causes of the nonlinear relation between smolts and resulting adults. As well, these nonlinear processes are the most likely ones to be circumvented in future stock management. Various authors discuss numerous processes that could cause compensatory mortality in the smolt-to-adult life stage. Competition for food might be important, particularly when fish are crowded in estuaries, as could numerical and functional responses of predators. Elson's (1962) work documents the aggregation of bird predators when fish prey abundances are high, and Meacham and Clark (1979) review similar significant predation phenomena by birds and fish on Alaskan sockeye smolts. Diseases could also be more prevalent and cause higher mortality at high fish abundance, but very little documentation on these variables exists for any single population. Competition for food could cause slower growth which would make fish vulnerable to predators for a longer span of time (Ricker 1954). Belford's (1978) model demonstrates the significance of this interaction of growth and predation processes for pink, chum (*O. keta*), and coho salmon. Disease can also interact with predation because diseased fish could be more vulnerable to predation (e.g. van Dobben 1952). At present, however, insufficient information has been gathered to show which mechanisms are most important in the case of Babine Lake sockeye salmon.

There are two possible explanations for the positive correlation between deviations in Babine Lake sockeye marine survival and abundance of juvenile pinks entering salt water in the same year as smolts: either smolts are feeding on these fry, growing larger and surviving better, or the pink fry are "buffering" sockeye smolts from predation and creating a depensatory mortality effect (Neave 1953). The first explanation is unlikely because salmon fry are rarely found in stomachs of juvenile salmon, regardless of whether they are sampled in freshwater (e.g. Semko 1954), coastal marine waters (Hartt 1980), or the Gulf of Alaska (e.g. LeBrasseur 1966). The second explanation seems more plausible. Juvenile pink and sockeye salmon migrate northward along a narrow, 37-km-wide band of water (Hartt 1980), and pink fry grow quickly enough that their size distributions in these waters considerably overlap those of sockeye as early as the end of July, and they have approximately the same mean lengths by mid-August (Hartt 1980; Hartt and Dell 1982). Healey (1978) finds similar evidence for faster growth of pinks and similar body size of pinks and sockeye by the fall in his sample years. Thus, single predator species such as seals and large fish could potentially have both sockeye and pink juveniles as prey and could be swamped by large combined populations, resulting in higher sockeye salmon survival. Unfortunately, few pink fry data for areas 1–10 exist, so it is not possible to test this idea by looking for a positive correlation between residuals in pink and Babine sockeye marine survivals.

If the predator swamping explanation is valid, then there are implications for enhancement. Persistent, high survival of Babine sockeye, for any given smolt abundance, may be achievable by manipulating appropriate enhanced pink populations to "fill in" expected low smolt production. To achieve this, enhancement efforts will have to be coordinated among species to ensure the most effective use of facilities.

An apparent dilemma is created by the observations here that increasing smolt abundances may have a deleterious effect on Babine sockeye smolt survival, but additional juvenile pink salmon in areas 1–10 increase survival of that stock. However, this dilemma can be resolved by hypothesizing that any within-population density dependence occurs during downstream migration or in early marine life, before pink juveniles overlap sockeye in physical distribution or before pinks grow to about the same size as smolts. Information on mortality processes in this early marine stage is sorely needed to test this hypothesis.

It is difficult to explain the inverse relation between adult pink returns in the year of sockeye smolt migration and residuals in marine survival of those smolts (Fig. 7). To my knowledge there is no documented evidence of adult pink salmon feeding on sockeye smolts at any time, or on the same food as sockeye smolts in coastal waters, although LeBrasseur (1966) shows that pinks on the high seas consume amphipods, as do juvenile sockeye. Other explanations seem even less plausible, so more information is needed on sockeye smolt and adult pink stomach contents in coastal waters and sockeye smolt marine growth to test the above possibilities.

To my knowledge, no other authors have clearly documented nonlinear marine survival in salmon, but Rogers (1980) and Mathews (1980) review evidence for density-dependent marine growth in salmon. Rogers' data on Bristol Bay sockeye in Alaska, for example, show that adult body size at a

given age is inversely related to abundance of fish in the run that year. He suggests that this density-dependent growth occurs in the last year of life as fish become concentrated on their homeward migration, a surprising view as most biologists feel that early life stages are most susceptible to competition.

Observation error data for smolt numbers should be gathered for other major salmon stocks because in the absence of such data, the functional regression relation between adults and smolts is best used. But Fig. 1 shows this relation can differ dramatically from the "corrected" relation. For similar reasons, Walters and Ludwig (1981) emphasize the importance of observation errors in the estimation of stock–recruitment relationships.

The lack of significant relation between smolt and adult abundances documented here for Babine Lake sockeye has major implications for further enhancement of this stock. The marginal rate of return per dollar invested in smolt production drastically decreases as smolt abundance increases. This is made clear in Fig. 2 where survival rates at high smolt numbers are roughly one quarter of those at low abundance. There is clearly a need to take this relation into account and to reanalyze the biological and economic costs and benefits of continuing to increase smolt production from Babine Lake. The reduced benefits of adult returns from large smolt migrations may not outweigh the additional costs associated with producing that abundance of smolts. On the other hand, continued efforts to boost smolt numbers may indeed be justified because it may cost very little to produce additional smolts, compared with capital and fixed operating costs of facilities which are already in place.

Findings here also demonstrate that it is unwise to assume a priori the existence of only one major limiting life stage in salmon population dynamics. Babine Lake enhancement facilities did successfully remove a bottleneck to fry production (Ginetz 1977), as they were designed to do, but the existence of compensatory and/or extremely variable mortality in the smolt-to-adult life stage means that increases in smolt abundance have caused smaller-than-expected changes in adult returns. Expectations in this case were based on a constant, density-independent survival rate. The Babine Lake sockeye population was already a relatively large, productive stock even before enhancement began, and so one might argue that results here may not be representative of most other salmon populations. However, calculations of expected benefits from future enhancement projects should at least take into account the possibility that other life stages besides freshwater habitat may be limiting.

Acknowledgments

Data were kindly provided by H. Smith, A. D. Anderson, and R. Kadowaki, Canadian Department of Fisheries and Oceans; Fred Wong, University of British Columbia; R. Gunsolus, Oregon Department of Fish and Wildlife; and C. Meacham, Alaska Department of Fish and Game. Howard Stiff assisted with statistical analyses. G. Geen, R. Routledge, and G. Steer made useful suggestions on the manuscript, as did P. Larkin and another reviewer. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada.

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Estimating Prey Preference by Predators: Uses of Various Indices, and a Proposal of Another Based on χ^2

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A really useful prey-selection index should be statistically testable. A 2 × 2 contingency table formulation provides a simple way to test the significance of any degree of selection at any sample size. An index ranging from −1 to +1, and zero-valued for no selection, can be simply derived from the chi-square formulation. Some conflicting usages in biological statistics texts are discussed, as well as the usefulness and limitations of other commonly used indices.

Key words: selection, index, chi-square, exact test, prey

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Pour être réellement utile, un indice de sélection des proies doit être statistiquement vérifiable. Un tableau de contingence de 2 × 2 est une méthode simple de tester n'importe quel degré de sélection avec échantillons de n'importe quelle taille. La formulation du chi-carré permet d'établir un indice de −1 à +1, avec valeur zéro dans le cas de non-sélection. Nous examinons certaines applications contradictoires relevées dans les manuels de statistiques biologiques, ainsi que l'utilité et les limitations d'autres indices communément utilisés.

Received March 12, 1981
Accepted March 12, 1982

Reçu le 12 mars 1981
Accepté le 12 mars 1982

PREDATOR diets often contain prey species in proportions different from those in the environment. A critical approach to analysis of food selection requires both a quantitative measure of these differences and a means of evaluating the reality of apparent departures from randomness. Even very high values of selection coefficients may be meaningless if based on too few data, and this applies both to data on gut contents (or prey removal) and those from the environment. Although many prey-selection indices have been proposed, few are provided with appropriate means for significance testing. I present here a simple index which is affected by both the apparent degree of prey selection and its statistical probability, and for which the selection probability can be easily calculated for any sample sizes. The formulation and behavior of other commonly used indices is then compared, together with the precision of the available statistical tests. Finally, I consider situations in which particular indices are appropriate.

Derivation of the Index

Several authors have proposed use of $2 \times j$ contingency chi-square statistics, where j = the number of species, as a test for overall randomness of feeding (Jolicoeur and Brunel 1966; Schneider 1981). However, to test for selection of a particular species it is necessary to reduce this to a 2 × 2 table, with all "other species" grouped (Cain and Sheppard 1954; Pearre 1974). The notation used in this paper is as follows:

	Species		
	A	Others	Total
Diet	a_d	b_d	$a_d + b_d = d$
Environment	a_e	b_e	$a_e + b_e = e$
Total	$a_d + a_e = a$	$b_d + b_e = b$	$a_d + a_e + b_d + b_e = n$

The most convenient method of evaluating χ^2 from these data is:

$$(1) \qquad \chi^2 = \frac{(a_d b_e - b_d a_e)^2 \cdot n}{a \cdot b \cdot d \cdot e},$$

with one degree of freedom.

Windell and Bowen (1978) suggested the use of an ordinary chi-square as a statistically meaningful index of food preference. However, this has three disadvantages for routine use. (1) It is always positive, hence does not signify whether an item is selected for or against. (2) It is unbounded, hence suffers some of the intuitive disadvantages discussed by Ivlev (1961) and Vanderploeg and Scavia (1979a). (3) The proportions found in environmental samples are taken as the expected values; hence the resultant chi-square incorporates no information about the precision of this estimate. This last objection is met by the use of the contingency form (above); the first two can be met by calculation as

$$V = \frac{a_d b_e - a_e b_d}{(a \cdot b \cdot d \cdot e)^{1/2}}$$

Printed in Canada (J6432)
Imprimé au Canada (J6432)

attributed to Yule by Kendall (1952). This varies between −1 and +1, but only reaches these extremes if two frequencies vanish. This was termed "absolute association" by Kendall (1952). He used the term "complete association" to denote the case in which only one frequency is zero. The same index may be derived starting from χ^2, as

$$V = \pm\left(\frac{\chi^2}{n}\right)^{1/2}.$$

The variance of V was also given by Yule (in Kendall 1952) as:

$$\text{(2) var } V = \frac{1}{n}\left[1 - V^2 + \left(V + \frac{V^3}{3}\right)\frac{(a_d - b_c)^2 - (b_d - a_c)^2}{(a\cdot b\cdot e\cdot d)^{1/2}} - \frac{3V^2}{4}\left\{\frac{(a_d + b_d - a_c - b_c)^2}{d\cdot e} - \frac{(a_d + a_c - b_d - b_c)^2}{a\cdot b}\right\}\right].$$

Yates' correction for continuity can be automatically incorporated in Eq. (1) by the modification:

$$\chi_c^2 = \frac{n\left(|a_d b_c - b_d a_c| - \frac{n}{2}\right)^2}{a\cdot b\cdot d\cdot e}$$

and from this, a Yates corrected analog of V can be derived:

$$\text{(3)} \quad C = \pm\left[\frac{\left(|a_d b_c - b_d a_c| - \frac{n}{2}\right)^2}{a\cdot b\cdot d\cdot e}\right]^{1/2}$$

or

$$C = \pm\left(\frac{\chi_c^2}{n}\right)^{1/2}.$$

Although the sign must be given by inspection (from $a_d b_c - a_c b_d$), the range and behavior of C is very similar to that of V, with the interesting difference that the limits (± 1) are only approached by C in absolute association as the remaining frequencies approach ∞.

Either of these indices may be easily tested for significance by various means:

1) The variance (Eq. 2) can be used to derive a "t"-test; however, this is by far the most laborious method.

2) $n\cdot|V|$ or $n\cdot|C|$ has the distribution of the normal deviate and may be tested either against tables of the normal deviate (z) or, if these are unavailable, against tables of areas under the normal curve or its tails. In the examples given here I have used the latter method to calculate probabilities of very high V and C.

3) The obvious alternative is the use of chi-square, which is also the square of the estimate of the normal deviate (above). I have used this in most of the examples to take advantage of the detailed tables of χ^2 with one degree of freedom given by Kendall (1952, appendix Tables 6 and 7).

4) If $n < 20$, or $20 < n < 40$, but the smallest expected frequency < 5, Snedecor and Cochran (1967) recommend use of Fisher's exact test. Although designed for 2×2 tables in which all marginal totals are fixed (Sokal and Rohlf 1969), this test has been shown to be optimal for the other models as well (Kendall and Stuart 1973).

In our notation, this test is

$$\text{(4)} \quad P = \frac{a!\,b!\,d!\,e!}{n!\,(a_d)!\,(a_c)!\,(b_d)!\,(b_c)!}.$$

This can be evaluated directly by use of a calculator or computer with factorial capacity, or from tables of logarithms of factorials (e.g. Rohlf and Sokal 1969). A BMDP (BioMedical Computer Programs 1979, p. 262) program can calculate Fisher's exact test (minimum expected value <20) as well as corrected and uncorrected χ^2. Alternatively the 2×2 matrix can be simply tested for significance (for specified limits of n) by shortcut tables (Mainland 1948; Siegel 1956; Finney et al. 1963; Langley 1971).

If testing a hypothesis such as "fish select larger zooplankton," it may be possible to use a one-tailed test, but in general testing selection of species A should be two-tailed, and this procedure is used in the examples (Tables 1–4). However, if using Fisher's exact test for very small numbers, it is dangerous to follow the recommendation of Siegel (1956, p. 103) or Sokal and Rohlf (1969, p. 595) to double the probability obtained from the first tail calculated. The tails are often asymmetrical and only probabilities lower than that of the observed distribution should be included. Shortcut procedures for determining which components to use are given by Croxton (1959, p. 277–278).

5) Windell and Bowen (1978) recommend the use of the G-statistic (Sokal and Rohlf 1969) for such testing. However, in this case it would involve extra labor, and it cannot be used if any frequency is zero.

Contingency Table Notation of Other Indices

Scott (1920) published the first known attempt to quantify selection, dividing the average number of each prey species per fish stomach by the number found in plankton hauls. In our notation this would give a number proportional to a_d/a_c. The ability to measure water volume sampled has enabled Clarke (1980), in what is perhaps the latest proposal, to standardize the plankton counts and express this statistic as "apparent search volume" (ASV) per fish for each prey species. ASV is not an index in the usual sense, i.e. it does not have a fixed value such as 0 or 1 indicating nonselective feeding, but despite criticism by Ivlev (1961) and Chesson (1978) appears to be a useful approach for some purposes, such as relating relative selection to known frequencies of encounter.

Shorygin (1939, cited by Ivlev 1961) and Hess and Swartz (1940) apparently independently derived the still-popular "forage ratio"; in our notation this is

$$\text{(5)} \quad \text{FR}_a = r_a/p_a = \frac{a_d}{d}\Big/\frac{a_c}{e} = \frac{e}{d}\cdot\frac{a_d}{a_c}$$

where r_a is the proportion of prey species a in the gut and p_a its proportion in the environment. However, this index is asymmetrical: ingestion which is less than proportional to the abundance of a species ("negative selection") is denoted by values between zero and one, with "positive selection" represented by the whole region from one to infinity. The value "one" denotes presence in the diet in exactly the same propor-

TABLE 1a. Variation of different indices as the numbers of organisms in the diet (*d*) and environment (*e*) change. Case 1: data chosen to give near-significant selection; Case 2: environmental counts twice as large as in Case 1; Case 3: "other species" twice as numerous as in Case 1; Case 4: all counts twice those of Case 1.

Text Eq. No.		Species[a]												
		Case 1			Case 2			Case 3			Case 4			
		A	Others	Σ	*A*	Others	Σ	*A*	Others	Σ	*A*	Others	Σ	Reference
	Diet	3	12	15	3	12	15	3	24	27	6	24	30	
	Environment	8	7	15	16	14	30	8	14	22	16	14	30	
	Σ	11	19	20	19	26	45	11	38	49	22	38	60	
	r_u		0.20			0.20			*0.11*			0.20		
	p_u		0.53			0.53			*0.36*			0.53		
	Index													
(5)	FR		0.375			0.375			*0.306*			0.375		Hess and Swartz 1940; Ivlev 1961
(6)	*E*		−0.455			−0.455			*−0.532*			−0.455		Ivlev 1961
(7)	*D*		−0.641			−0.641			−0.641			−0.641		Jacobs 1974
(8)	*Q*		0.219			0.219			0.219			0.219		Jacobs 1974
(9)	*L*		−0.333			−0.333			*−0.253*			−0.333		Jolicoeur and Brunel 1966
(10)	α		0.179			0.179			0.179			0.179		Chesson 1978
(11)	*W*		0.302			*0.255*			0.302			0.302		Vanderploeg and Scavia 1979a, b
(12)	*E**		−0.248			*−0.325*			−0.248			−0.248		Vanderploeg and Scavia 1979a
(13)	β_N		0.242			*0.217*			0.242			0.242		Manly 1974
(1)	*V*		−0.346			*−0.318*			*−0.301*			−0.346		This paper
(3)	*C*		−0.277			*−0.270*			*−0.252*			*−0.311*		This paper

[a]Italicized values differ from values for Case 1 (≡ "instability").

TABLE 1b. Significance tests for L, V, and C.

Index	Test	Case 1	Case 2	Case 3	Case 4
		True probability[a]			
		0.1281	0.0542	0.0455	0.0150
L	t	2.02	2.42	2.12	2.855
	Probability interval[b]	0.01–0.05	0.02–0.01	0.05–0.025	0.01–0.005
V	χ^2	3.589	4.555	4.440	7.177
	Probability interval[c]	0.061–0.058	0.034–0.032	0.036–0.033	0.0077–0.0073
C	χ^2	2.297	3.291	3.108	5.813
	Probability interval[c]	0.129–0.121	0.074–0.069	0.078–0.074	0.016–0.015

[a]From Fisher's exact test, two tails calculated separately (Eq. 4).
[b]Two-tailed "Student's-t" test, df = $n-2$ (Strauss 1979); Rohlf and Sokal 1969, Sect. Q: 'Critical values of Student's t-distribution.'
[c]Kendall (1952), Appendix Table 7: 'Distribution function of χ^2 for one degree of freedom for values of χ^2 from $\chi^2 = 1$ to $\chi^2 = 10$ by steps of 0.1.'

tions as in the environment, otherwise known as "no selection" or "random feeding." Jacobs (1974) pointed out that the asymmetry could be corrected by using a logarithm of FR (range $-\infty$ to $+\infty$, with random feeding = 0). Ivlev's (1961) solution was "electivity,"

$$(6)\quad E_a = \frac{r_a - p_a}{r_a + p_a} = \frac{\frac{a_d}{d} - \frac{a_e}{e}}{\frac{a_d}{d} + \frac{a_e}{e}} = \frac{a_d \cdot e - a_e \cdot d}{a_d \cdot e + a_e \cdot d}$$

ranging from -1 to $+1$. This has undoubtedly been the most popular index since then, but has recently come under criticism. Boyd (1976) and Vanderploeg and Scavia (1979a) observed that E for size selection varied with the shape of the offered size spectrum. Pearre (1973) and Lechowicz (1982) noted that E is incapable of fairly assessing selection of rare prey, and Strauss (1979) showed that the entire sampling distribution is skewed. He derived confidence intervals for E and FR and showed that these were extremely wide when $p_a(=\frac{a_e}{e})$ is small. Jacobs (1974), noting that changes of prey availability could lead to changes in E during the course of an experiment, proposed an analog of E,

$$(7)\quad D_a = \frac{r_a - p_a}{r_a + p_a - 2r_d \cdot p_a} = \frac{a_d \cdot e - a_e \cdot d}{a_d \cdot e + a_e \cdot d - 2a_d a_e}$$

and one for FR,

$$(8)\quad Q_a = \frac{r_a(1 - p_a)}{p_a(1 - r_a)} = \frac{a_d \cdot e - a_d \cdot a_e}{a_d \cdot d - a_d \cdot a_e}.$$

Jolicoeur and Brunel (1966) and Strauss (1979) independently proposed an index which is essentially the numerator of E; in Strauss' notation this is:

$$(9)\quad L_a = r_a - p_a, \text{ or } \frac{a_d}{d} - \frac{a_e}{e}.$$

L is intended to be easy to calculate and linear, and Strauss provided an expression for its variance so that its significance could be easily tested.

Chesson (1978) and Paloheimo (1979) proposed an index based on a constant preference coefficient discussed by O'Neill (1969). It can be expressed as a forage ratio normalized by the sum of all forage ratios; for a three-species case this is, in our notation:

$$(10)\quad \alpha_a = \frac{r_a}{p_a} \Big/ \sum_{i=1}^{3} \left(\frac{r_i}{p_i}\right) = \frac{e}{d} \cdot \frac{a_d}{a_e} \Big/ \frac{e}{d}\left(\frac{a_d}{a_e} + \frac{b_d}{b_e} + \frac{c_d}{c_e}\right) = \frac{a_d}{a_e} \Big/ \left(\frac{a_d}{a_e} + \frac{b_d}{b_e} + \frac{c_d}{c_e}\right).$$

As noted by Clarke (1980), this is also equal to ASV normalized by the sum of ASVs.

Vanderploeg and Scavia (1979a, b) proposed a very similar index, but with a primary definition of

$$W_i = f_i \Big/ \sum_{i=1}^{j} f_i$$

where f_i = the proportion of species i eaten, or

$$f_i = \frac{(\text{initial standing crop } i - \text{final standing crop } i)}{\text{initial standing crop } i}.$$

In our notation, then,

$$f_a = \frac{(a - a_e)}{a} = \frac{a_d}{a}.$$

So that W, again for three species, is

$$(11)\quad W_a = \frac{a_d}{a} \Big/ \left(\frac{a_d}{a} + \frac{b_d}{b} + \frac{c_d}{c}\right).$$

These authors (1979a) also proposed an index analogous in range and central value to E:

$$(12)\quad E_i^* = \frac{W_i - \left(\frac{1}{j}\right)}{W_i + \left(\frac{1}{j}\right)}.$$

The form of α and W had been anticipated by Manly et al. (1972) and Manly (1974), who proposed two new indices. For three species, the one intended for use with field data is:

$$(13)\quad \beta_{N\alpha} = \log(1 - f_a) \Big/ \sum_{i=1}^{3} \log(1 - f_i) = \log\left(\frac{a_e}{a}\right) \Big/ \left[\log\left(\frac{a_e}{a}\right) + \log\left(\frac{b_e}{b}\right) + \log\left(\frac{c_e}{c}\right)\right].$$

TABLE 2a. Effect of lengthened grazing time on selection, with and without prey replacement: data chosen to fit Manly's (1972) tables for variance of β_N.[a]

	Species								
	Case 1: original set			Case 2: no replacement			Case 3: with replacement		
	A	Others	Σ	*A*	Others	Σ	*A*	Others	Σ
Diet	6	14	20	12	28	40	12	28	40
Environment	24	16	30	18	2	20	24	16	40
Σ	30	30	60	30	30	60	36	44	80
r_a		0.3			0.3			0.3	
p_a		0.6			*0.9*			0.6	
Index									
FR		0.500			*0.333*			0.500	
E		−0.333			*−0.500*			−0.333	
D		−0.555			*−0.909*			−0.555	
Q		0.285			*0.0476*			0.285	
L		−0.300			*−0.600*			−0.300	
α		0.222			*0.0454*			0.222	
W		0.300			0.300			*0.344*	
*E**		−0.250			−0.250			*−0.185*	
β_N		0.262			*0.159*			*0.286*	
V		−0.283			*−0.566*			*−0.302*	
C		−0.248			*−0.530*			*−0.276*	

[a]Italicized values differ from values for Case 1.

TABLE 2b. Significance tests for *L*, β_N, *V*, and *C*.

		Case 1	Case 2	Case 3	
		True probability[a]			
Index	Test	0.0539	0.000019	0.0129	Reference
L	*t*	2.336	6.076	2.828	Strauss (1979)
	Probability interval[b]	0.05−0.02	<0.001	0.01−0.001	
β_N	*t*	2.519	2.306	3.255	Manly (1972)
	Probability interval[b]	0.02−0.01	0.05−0.02	0.01−0.001	
V	χ^2	4.800	19.20	7.273	
	Probability interval	0.0285[c]	0.000012[d]	0.0073−0.0069[c]	
C	χ^2	3.675	16.88	6.111[c]	
	Probability interval	0.0578−0.0544[c]	0.000040[d]	0.0135−0.0128[c]	

[a]Fisher's exact test (as before).
[b]"Student's-*t*" test (as before).
[c]Kendall (1952), Appendix Table 7 (as before).
[d]Croxton (1959), Appendix IV: 'Areas in two tails of the normal curve at selected values of x/s or x/σ from the arithmetic mean.'

This was denoted by Manly (1974) as "$\hat{\beta}_i$," for experiments without prey replacement. The second index, which I will call "β_R," is intended only for experiments in which prey are replaced as eaten:

$$\beta_{Ra} = \frac{a_d}{x_a} \Big/ \left(\frac{a_d}{x_a} + \frac{b_d}{x_b} + \frac{c_d}{x_c}\right)$$

where x_i represents the number of species i available at the beginning of the experiment. Because this number actually equals line "*e*" of our table if prey are always replaced, this index is identical with α (Eq. 10) under these conditions. However, if used for field data (for which Manly did not intend it), it would appear to be equivalent to *W* (Eq. 11). Because of the ambiguity I shall refer to α and *W* rather than to β_R in the text and tables, but it should be noted that the condition of prey replacement is similar to one in which *n* is very large and/or only a small proportion of available prey is consumed ($e \rightarrow n$). For these conditions Manly (1974) has shown that β_N converges with β_R and also, especially as $\frac{a_d}{a_e}$ decreases, *W* converges with α (Table 1). However, for small samples β_N, *W*, and α can differ considerably (Tables 1 and 2).

Manly (1974) provided equations for estimating the variance of either β_R or β_N; the latter especially is extremely

TABLE 3a. Behavior of various indices in conditions of complete association (see text).

	Species								
	Case 1: near significance			Case 2: no selection			Case 3: significant		
	A	Others	Σ	*A*	Others	Σ	*A*	Others	Σ
Diet	0	16	16	2	31	33	10	20	30
Environment	4	12	16	0	23	23	10	0	10
Σ	4	28	32	2	54	56	20	20	40
r_a		0			0.061			0.33	
p_a		0.25			0			1.00	
Index									
FR		0			∞			0.333	
E		−1.00			1.00			−0.500	
D		−1.00			1.00			−0.667	
Q		0			∞			0	
L		−0.250			0.061			−0.667	
α		0			∞/∞			0	
W		0			0.635			−0.333	
*E**		−1.00			0.119			−0.200	
β_N		0			$-\infty$			$-\infty$	
V		−0.378			0.161			−0.577	
C		−0.283			0.063			−0.520	

TABLE 3b. Significance tests for measures of selection.

		Case 1	Case 2	Case 3
		True probability[a]		
Index	Test	0.101	0.507	0.00044
L	*t*	2.31	0.254	7.75
	Probability interval[b]	0.05−0.02	0.9−0.8	<0.001
V	χ^2	4.571	1.45	13.33
	Probability interval	0.034−0.032[c]	0.237−0.221[c]	0.00032−0.00022[d]
C	χ^2	2.571	0.221	10.8
	Probability interval	0.114−0.107[c]	0.639−0.632[c]	0.0014−0.00097[d]

[a]Fisher's exact test (as before).
[b]"Student's-*t*" test (as before).
[c]Kendall (1952), Appendix Table 7 (as before).
[d]Croxton (1959), Appendix IV (as before).
[e]Kendall (1952), Appendix Table 6. 'Distribution function of χ^2 for one degree of freedom for values of χ^2 from $\chi^2 = 0$ to $\chi^2 = 1$ by steps of 0.01.'

complex and could only be routinely solved with a computer. Also, as he warns, it can only be considered valid if among other conditions all expected values of numbers eaten and remaining exceed 5 and all observed values exceed 10. However, Manly (1972) provided tables for interpolation of standard deviations for the two-species case, with total n (number of individual prey) from 10 to 80, total numbers of species j from 10 to 50% of n, and proportions eaten between 30 and 70%. This appears to be the first instance of an author proposing means of testing the significance of a new index.

Lechowicz (1982) derived an elegant means of graphical representation of the behavior of most of the indices discussed here. Unfortunately it is not possible to present *V* or *C* in this manner as they do not rely only on r_a and p_a, but are essentially four-dimensional. In the next section the values of the indices for various combinations of synthetic data are compared. The probability of nonrandomness in each "data set" has been directly calculated by Fisher's exact method (see above). For those indices which are provided with simple significance tests, including data which will fit Manly's (1972) tables, estimates of the significances are also calculated and compared.

Comparison of the Indices in Mock Experiments

Under the conditions imposed in Table 1, both of Jacob's (1974) indices and that of Chesson (1978) are completely stable, i.e. retain the same numerical value. Increasing the numbers of alternate prey (Case 3) decreased FR, *E*, *L*, *V*, and *C*; this may not be unreasonable because of the decreased

TABLE 4a. Behavior of various indices in conditions of absolute association (see text).

	Species								
	Case 1: near significance			Case 2: significant			Case 3: high n		
	A	Others	Σ	A	Others	Σ	A	Others	Σ
Diet	0	2	2	16	0	16	0	80	80
Environment	5	0	5	0	16	16	100	0	100
Σ	5	2	7	16	16	32	100	80	180
r_a		0			1.0			0	
p_a		1.0			0			1.0	
Index									
FR		0			∞			0	
E		−1.00			1.00			−1.00	
D		−1.00			1.00			−1.00	
Q		0			∞			0	
L		−1.00			1.00			−1.00	
α		∞/∞			∞/∞			∞/∞	
W		0			∞			0	
E^*		−1.00			1.00			−1.00	
β_N		−∞			−∞			−∞	
V		−1.00			1.00			−1.00	
C		−0.650			0.938			−0.989	

TABLE 4b. Significance tests for measures of selection.

		Case 1	Case 2	Case 3
		True probability[a]		
Index	Test	0.0952	$3.3 \cdot 10^{-9}$	$6.7 \cdot 10^{-53}$
L	t	∞	∞	∞
	Probability interval	0	0	0
V	χ^2	7.00	32.0	180
	Probability interval	~0.00185[b]	$2.1 \cdot 10^{-8}$–$1.2 \cdot 10^{-8}$[c]	$\ll 10^{-12}$[c]
C	χ^2	2.96	28.13	175.97
	Probability interval	0.089–0.083[b]	$\sim 1.2 \cdot 10^{-6}$[c]	$\ll 10^{-12}$[c]

[a]Fisher's exact test (as before).
[b]Kendall (1952) Appendix Table 7 (as before).
[c]Croxton (1959) Appendix IV (as before).

proportions of A. Increasing the counts from the environment (Case 2), which should only alter the precision of estimation, caused a decrease in W (and hence E^*), V, and C. C increased as the numbers of all components in the table increased together (Case 4); all other indices were constant. Table 2a shows the effect of grazing increasing numbers of prey with and without replacement. Case 2 is derived by doubling the amounts taken in Case 1, as might happen in a longer experiment, while retaining fixed total prey. The apparent stability of W in Case 2 is deceptive, for in this case selection on species A must have decreased as a result of its increasing proportion (p_a) in the environment. E, D, and E^* share the disadvantage (Strauss 1979) of being always −1 when a_d (the number of species A in the diet) is zero, in which case FR, Q, α, β_N, and W are zero (Table 3). Alpha (α) goes to zero if either a_d or $b_e = 0$, one if $b_d = 0$, and is undefined if $a_e = 0$ (Table 2), and in all cases of absolute association (Table 4).

This problem was also considered by Manly (1972), but is probably insoluble with this type of index. While complete or absolute association may be difficult to interpret in terms of predator behavior, either can occur in field data and they cannot be dismissed unless statistically insignificant.

L (Jolicoeur and Brunel 1966) appears to be generally well behaved, its chief difficulty being in significance testing either with low n or extreme proportions (Strauss 1979). For absolute association, the t-test for L is always infinite. It is less conservative than χ^2_c, which is usually the closest to, but more conservative than, Fisher's exact test (Tables 1b–4b, 5).

Sokal and Rohlf (1969) recommend against the use of Yates' correction of χ^2 for all but very small n, but Snedecor and Cochran (1967, p. 211) feel that it should be used routinely. Although clearly less powerful than the uncorrected χ^2_v, the Yates-corrected version gave a much better approxi-

mation of the probability in most cases tested (1b–4b, 5), and Type II errors are undoubtedly less serious than Type I in most selection studies. This plus its unique ability to distinguish between cases of absolute association at different values of n make C a clear choice over V as an index of prey selection. If an index more strictly proportional to probability is desired, $C \cdot n$, the Yates-corrected estimate of the normal deviate, would serve.

Discussion

The sensitivity of W (Vanderploeg and Scavia 1979a, b) to experimental duration or prey digestion (Tables 1, 2) suggest that α (Chesson 1978; Paloheimo 1979) may be the more useful. β_N (Manly 1974, as β_i for experiments without replacement) has vices and virtues similar to both, but is slightly more difficult both to calculate and interpret. Although all of these indices behave erratically in complete or absolute association (Tables 3 and 4), these conditions can generally be avoided at least in laboratory experiments, and these indices are particularly valuable for such circumstances. However, neither α nor β_N can be directly tested and α becomes nonlinear when any low frequencies are encountered (Manly 1974; Chesson 1978). Although no tests have been proposed for W, these should be equivalent to tests for β_R at large sample sizes.

TABLE 5. Comparison of tests of significance of selection in small samples: Case 1: data chosen to give result significant by t-test of L but not by exact test. Case 2: data chosen for significant results at small n with no zero frequencies.

		Species					
		Case 1			Case 2		
		A	Others	Σ	A	Others	Σ
Diet		8	7	15	4	2	6
Environment		2	13	15	1	9	10
Σ		10	20	30	5	11	16
r_a			0.53			0.67	
p_a			0.13			0.10	
True probability[a]			0.0502			0.0357	
Estimate of exact test by 2× (first tail)			0.0502			0.0714	
Index	Test						
L			0.400			0.618	
	var. L[b]		0.0243			0.0460	
	t		2.57			2.88	
	Prob. interv.		0.02–0.01			0.02–0.01	
β_N			0.789			0.889	
	$\sigma\beta_N$[c]		0.151			0.124	
	t		6.76			7.20	
	Prob. interv.		<0.001			<0.001	
V			0.424			0.592	
	var. V[d]		0.0251			0.0297	
	t		2.68			3.43	
	Prob. interv.		0.02–0.01			0.005–0.001	
	χ^2		5.400			5.605	
	Prob. interv.[e]		~0.0201			0.018–0.017	
	G[f]		5.683			5.735	
	Prob. interv.[e]		0.018–0.017			0.017–0.016	
C			0.354			0.453	
	var. C[d]		0.0276			0.0660	
	t		2.13			1.76	
	Prob. interv.		0.05–0.025			~0.10	
	χ^2		3.750			3.278	
	Prob. interv.[e]		0.054–0.051			0.074–0.069	
	G[f]		3.880			3.270	
	Prob. interv.[e]		0.051–0.048			0.074–0.069	

[a]Fisher's exact test (as before).
[b]Variance from Strauss (1979).
[c]Interpolation from Manly (1972) Table 2: 'Standard error for single β estimates for experiments with values of N ranging from 10 to 80.'
[d]Variance calculated by text equation 2.
[e]Kendall (1952) Appendix Table 7 (as before).
[f]G statistic: Sokal and Rohlf (1969).

L (Jolicoeur and Brunel 1966) is appealing because of its ease of calculation and general good behavior, as well as the provision of a simple significance test by Strauss (1979). Strauss, however, warns against use of the t-test at small values of d or e combined with extremes of r_a or p_a; and indeed it appears to yield ample opportunity for Type I errors under these conditions (Tables 1b–4b, 5). The reason, as Strauss points out, is that proportions can only be considered approximately normal near the middle of their ranges. It does not appear possible to cure this defect by a "normalizing" transformation such as the arcsine, so the limitations for testing of this index are rather similar to those of the indices described above. Lechowicz (1982) found that preference rankings obtained by its use on field data disagreed with rankings by the other indices.

The indices which we have been discussing are of two general types. FR, E, D, Q, L, and C reflect the results of the predator–prey interaction in the particular circumstances observed. The second type, exemplified by W, E^*, α, and β_N, is supposed to measure an invariant degree of preference on the part of the predator. Such an index, though worthwhile in itself, by definition contains less information than one of the first group. For example, analysis of time- and depth-related variations in E for various prey of Chaetognatha led to a hypothesis about the proximal cause of vertical migration (Pearre 1973). A more robust index such as W, E^*, α, or β_N might have masked much of this variation. Perhaps ideally we should use two indices, one such as α or E^* entirely dependent on the predator, and the other entirely dependent on the environment, perhaps obtained by subtraction from FR or E; from Table 1 it appears that the absolute differences trend in the proper direction. However, partitioning the significance tests (once we have derived them) may be more complex. Other problems such as the role of prey defenses (Borutskii 1960; Ivlev 1961; Pearre 1974) or patchiness (Bullen 1908; Ivlev 1961; O'Brien and Vinyard 1974) may well prove intractible for any type of index.

Lechowicz (1982) compared the formal properties of most of these indices to his criteria for an ideal prey-selection index. He postulated that such an index should: (a) be zero-valued for no selection ($r_a = p_a$); (b) be symmetrical about this value; (c) range between +1 and −1; (d) change linearly with changes in prey utilization; (e) not be sensitive to sampling errors, e.g. for rare prey species; (f) be statistically testable; and (g) be stable (= more or less constant valued for the same prey species).

As Lechowicz remarked, no single index yet proposed satisfies all of these criteria. Although C is not linear (d) and is, by its nature, the least stable of all (g), it still appears to satisfy more nearly these criteria than any of the other indices.

Conclusion

Among the indices reviewed here, different ones seem appropriate for different purposes, and the "best" method of calculating prey preference depends not only on ecological or mathematical theory, but on the question we wish to ask. Although claiming no particular ecological pedigree, C is related to the probability of selection (at any n), varies between −1 and +1, and is simply and precisely testable for any frequencies or sample sizes. Whenever low frequencies of prey species are expected or found, and any other time that the statistical evaluation of apparent nonrandomness is important, C may be a good choice of index.

Acknowledgments

I thank C. A. Field (Department of Mathematics, Dalhousie University) for invaluable advice on the use of test procedures. S. R. Kerr, J. A. Koslow, R. A. Myers, and P. Pepin have critically reviewed the manuscript in several versions and provided immense help. Publication costs were funded by a grant from the Chesapeake Bay Foundation.

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Variation of Hepatic Ethoxycoumarin *O*-de-ethylase Activity with Body Weight and Other Factors in Brook Trout (*Salvelinus fontinalis*)

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ADDISON, R. F., AND D. E. WILLIS. 1982. Variation of hepatic ethoxycoumarin *O*-de-ethylase activity with body weight and other factors in brook trout (*Salvelinus fontinalis*) Can. J. Fish. Aquat. Sci. 39: 924–926.

Data from several previous experimental studies of hepatic mixed function oxidase (MFO) activity were reexamined to study the possible relationship between ethoxycoumarin *O*-de-ethylase activity and body weight and other factors in "uninduced" brook trout (*Salvelinus fontinalis*). Weight-specific *O*-de-ethylase activity was inversely related to body weight; regression of the log transformation of the total hepatic *O*-de-ethylase activity on log liver weight yielded a regression coefficient of 0.850 ± 0.047 close to the value of −0.75 expected for a relationship based on overall metabolic rate. The regression coefficient was significantly higher in males than in females. Multiple regression analysis showed *O*-de-ethylase activity to be positively correlated with both microsomal protein and cytochrome P-450 content. The data confirm that size (or age) is a factor to be eliminated in any biological effects monitoring program based on MFO assays.

Key words: mixed function oxidases, ethoxycoumarin *O*-de-ethylase, brook trout, *Salvelinus fontinalis*; hepatic mono-oxygenases

ADDISON, R. F., AND D. E. WILLIS. 1982. Variation of hepatic ethoxycoumarin *O*-de-ethylase activity with body weight and other factors in brook trout (*Salvelinus fontinalis*) Can. J. Fish. Aquat. Sci. 39: 924–926.

Les résultats de plusieurs expériences antérieures sur l'activité de la fonction mixte hépatique oxydase (FMO) ont été réexaminés en vue de découvrir une relation possible entre l'activité de *O*-déséthylase de l'éthoxycoumarine et le poids corporel et autres facteurs chez l'omble de fontaine (*Salvelinus fontinalis*) « non-induits ». L'activité de *O*-déséthylase spécifique au poids est en relation inverse du poids corporel; la régression du log de la transformation de l'activité de *O*-déséthylase hépatique totale sur le log du poids du foie donne un coefficient de 0,850 ± 0,047, ce qui se rapproche de la valeur de 0,75 à laquelle on s'attend dans une relation fondée sur un taux métabolique général. Le coefficient de régression est significativement plus élevé chez les mâles que chez les femelles. Une analyse de régression multiple démontre que l'activité de *O*-déséthylase est en corrélation positive avec la teneur en protéines des microsomes et du cytochrome P-450. Ces données confirment que la taille (ou l'âge) est un facteur à éliminer dans tout programme de surveillance d'effets biologiques fondé sur des analyses de MFO.

Received May 11, 1981
Accepted February 19, 1982

Reçu le 11 mai 1981
Accepté le 19 février 1982

WE have been studying the hepatic mixed function oxidase (MFO) enzymes in fish to define their response to environmental contaminants and to explore the use of MFO induction in "biological effects monitoring" (cf. Anon 1980; McIntyre and Pearce 1980). In the course of this work, we have noticed that some indices of MFO induction in brook trout (*Salvelinus fontinalis*) varied with factors other than the extent of experimental exposure to inducers; in particular, MFO activity appeared to be inversely related to body weight (Addison et al. 1981). This has prompted us to reexamine our accumulated data to define the relationship between MFO activity and body weight, and possibly other variables.

Procedures — We reexamined data which had formed the basis of several previous publications (Addison et al. 1977, 1978, 1979, 1981), and we included some unpublished results. We used data derived only from fish which had been hatched from Antigonish, N.S., domestic stock and reared at Coldbrook Hatchery, Kentville, N.S. The fish had been obtained from the hatchery at different sizes, and they were kept in our laboratory's freshwater tanks for varying periods. During their stay in the laboratory they were fed a commercial fish diet (Ewoz) and were usually acclimated to temperatures

Printed in Canada (J6496)
Imprimé au Canada (J6496)

TABLE 1. Summary of data analyzed as described in text (data as mean ± SD (no of samples)).

Source of data	Age of fish (yr)	Date killed and maturity	Body wt (g)	*O*-de-ethylase activity (nmol product formed·g liver^{-1}·min^{-1})
Addison et al. 1978	1.3	May 1977; immature gonads	63.3±15.4 (6)	2.12±0.26 (6)
Addison et al. 1979	1.0	Mar 1978; immature gonads	131.7±20.9 (6)	1.37±0.37 (6)
Unpublished data	1.2	May 1978; immature or maturing gonads	148.1±12.6 (8)	1.55±0.19 (8)
Addison et al. 1981	1.5	Oct. 1978; maturing gonads	243.4±50.6 (24)	1.45±0.30 (24)
Unpublished data	1.5	June 1980; maturing gonads	283.7±60.0 (8)	1.21±0.34 (8)

of 10−15°C. At the time of analysis, they varied from immature to gonadally maturing. We examined data from only those fish which had been used as controls in our experiments; we had not intentionally exposed them to potential inducers of MFO activity. MFO assays are described in Addison et al. (1977); these were substantially similar from experiment to experiment throughout the review period. In all experiments, appropriate standards were analyzed so that data from one experiment to the next were comparable. Table 1 summarizes the data which were analyzed.

We first analyzed data from 52 male and female fish and investigated the relationship between MFO activity, as exemplified by ethoxycoumarin *O*-de-ethylase activity, and body weight. We then examined the possible variation of this regression between sexes, using data from 40 of the 52 fish as some data were missing for some fish. Finally, we examined the possible dependence of *O*-de-ethylase activity on other factors by multiple regression analysis, using 32 male and female fish for which appropriate data were available.

Results and discussion — Ethoxycoumarin *O*-de-ethylase activity per unit weight of liver was correlated negatively with body weight for 52 male and female fish. The best least squares fit for the regression was given by the log transformation which had the form:

$$(1) \quad \ln y = -0.211 \ln x + 1.47 \qquad (r = 0.414)$$

where y is enzyme activity as nmol product formed·g liver^{-1}·min^{-1} and x is body weight (g). The regression coefficient (-0.211 ± 0.066, mean ± SE) was significantly different from zero ($P = 0.002$). This regression depended on the inclusion of data from the smaller fish: if fish weighing less than 100 g were omitted from the analysis, though there was no objective reason to omit them, the regression coefficient did not differ significantly from zero. Thus, although *O*-de-ethylase activity was obviously related inversely to body weight, the regression of the log transformations may not provide the best description of the relationship. However, as it is a simple model used frequently in studies of metabolic activity, we have used it in the analyses described below.

When total hepatic MFO activity (y of Eq. (1) multiplied by liver weight) was regressed on liver weight, the following equation describing weight-specific activity was derived (for the log transformations):

$$(2) \quad \ln y_1 = 0.850 \ln x_1 + 0.531 \qquad (r = 0.93)$$

where y_1 is total hepatic MFO activity as nmol product formed·min^{-1}·liver^{-1} and x_1 is liver weight (g). The regression coefficient (0.850 ± 0.047, mean ± SE) differed significantly from zero ($P < 0.001$). Total MFO activity (y_1 of equation 2) regressed on total body weight yielded a regression coefficient of 1.14 ± 0.08 (mean ± SE), as liver weight was an increasing fraction of total body weight in larger fish.

Separate calculations analogous to those which yielded Eqn. (2) for groups of 21 male and 19 female fish yielded regression coefficients of 0.99 ± 0.11 and 0.89 ± 0.09, mean ± SE, respectively. These differed significantly ($P < 0.05$) indicating that in male fish, larger livers had more activity than in females. Regressions of liver weight on total body weight did not differ between the sexes.

Multiple regressions of *O*-de-ethylase activity in 32 male and female fish showed it to be positively significantly correlated with liver protein and cytochrome P-450 content.

The regression coefficient in Eqn. (2) is close to the value of ~0.75 which we would expect if weight-specific MFO activity were associated solely with overall metabolic rate (e.g. Prosser 1961). However, although MFO activity may well reflect overall metabolic rate, it also responds to other natural variables such as sexual maturity (Forlin 1980; Stegeman and Chevion 1980) and induction by certain chemicals. These analyses show that the form of the relationship between MFO activity and liver weight also varies with sex. The emergence of protein and cytochrome P-450 content as variables closely associated with *O*-de-ethylase activity is consistent with current views of the operation of MFO systems (e.g. Parke 1975).

MFO activity is known to vary in a complex way with age and maturity and therefore size in various mammals (Dickerson and Basu 1975). We believe that these results are the first direct evidence that similar relationships exist in fish, though Forlin (1980) has found the experimental induction of certain MFO enzymes to differ in 6-mo and 18-mo-old

trout.

These observations confirm that size, or possibly age, is a variable which should be eliminated in any attempt to monitor the sublethal effects of contaminants via MFO activity.

Acknowledgments — We thank Dr S. R. Kerr for his comments on the manuscript, and Mrs C. A. Eaton for information about the origin of the fish.

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Myxosoma cerebralis: Inactivation of Spores by Hot Smoking of Infected Trout

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WOLF, K., AND M. E. MARKIW. 1982. *Myxosoma cerebralis*: inactivation of spores by hot smoking of infected trout. Can. J. Fish. Aquat. Sci. 39: 926–928.

We processed 2-yr-old rainbow trout (*Salmo gairdneri*) and brook trout (*Salvelinus fontinalis*) with clinical whirling disease, which is caused by *Myxosoma cerebralis*, in one of the following ways: iced, iced and brined, or iced, brined, and hot smoked at 66°C for 40 min. Skeletal elements of each group were added to tanks containing soil samples from an aquatic environment free of *M. cerebralis* and aged for 4 mo at 12.5°C. Following this we assayed for infectivity by holding susceptible rainbow trout fry for 3 mo in the tanks of aquatic soil and skeletal elements. The fry were then examined for *M. cerebralis* spores. Spores were found in the fry from tanks that had received iced and iced and brined samples, but not in fry from the tanks containing hot smoked tissue, healthy tissue, or no tissue — the last two being negative controls.

Key words: whirling disease, *Myxosoma cerebralis*, heat inactivation, hot smoking

WOLF, K., AND M. E. MARKIW. 1982. *Myxosoma cerebralis*: inactivation of spores by hot smoking of infected trout. Can. J. Fish. Aquat. Sci. 39: 926–928.

Des truites arc-en-ciel (*Salmo gairdneri*) et des ombles de fontaine (*Salvelinus fontinalis*) de 2 ans, atteints de la maladie clinique gyratoire causée par *Myxosoma cerebralis*, ont été soumis à l'un des traitements suivants : glaçage; glaçage et saumurage; ou glaçage, saumurage et fumage à chaud à 66°C pendant 40 min. Des éléments squelettiques de chaque groupe furent ajoutés à des bassins contenant des échantillons de sol provenant d'un environnement aquatique libre de *M. cerebralis* et laissés pendant 4 mois à 12,5°C. On a ensuite procédé à des essais de contamination en maintenant des alevins de truite arc-en-ciel pendant 3 mois dans des bassins contenant des échantillons de sol et des éléments squelettiques. Les alevins ont ensuite été examinés en vue d'y détecter la présence de spores de *M. cerebralis*. On a

Printed in Canada (J6721)
Imprimé au Canada (J6721)

trouvé des spores chez les alevins maintenus dans les bassins contenant les échantillons glacés, et glacés saumurés, mais non chez ceux contenant des tissus fumés à chaud, des tissus sains, ou absence de tissus — ces deux derniers étant des témoins négatifs.

Received November 12, 1981
Accepted March 10, 1982

Reçu le 12 novembre 1981
Accepté le 10 mars 1982

Myxosoma cerebralis, the myxosporidan protozoan that is the etiologic agent of salmonid whirling disease, produces infections ranging from subclinical to lethal. In addition, fish that survive light to intermediate infection are lifelong carriers. If such infected fish are transported to, or stocked in, waters where the disease has not occurred, they have the potential of introducing the pathogen. Accordingly, in some places fish health authorities have effected measures directed toward eradicating the parasite.

In the United States whirling disease is known from limited areas of the Northeast and West (Wolf 1974). The condition has been identified at two federal and several state hatcheries and also at commercial facilities. Where eradication was attempted, clean-up operations resulted in destruction and burial of several million fry and fingerlings and tens of thousands of kilos of fish of edible size.

In Europe, whirling disease is controlled by sanitary measures and rearing susceptible fish to the advanced fingerling stage in uncontaminated spring or well water. The older and more resistant fish may then be held in contaminated waters without undue loss or disfigurement, even though they become subclinically infected and asymptomatic carriers. Such infected fish appear normal, but fish health regulations in several countries prevent their importation.

Myxosoma cerebralis poses no threat to human health; in fact, it is likely that many of the trout produced for human food in Europe have subclinical infections with whirling disease. It has been assumed that cooking kills the spores of *M. cerebralis*, just as it does the trichina worm in pork, and other parasites in meat and meat products.

It is unknown if smoking of salmonids inactivates the spores of *M. cerebralis*. If smoking were effective, infected fish destined for destruction in eradication procedures could be used as human food. Also, the nations now barring fresh or live fish with *M. cerebralis* infection might well reduce the strictness of their regulations and allow importation of an appropriately smoked product.

Materials and methods — Fourteen 2-yr-old rainbow trout (*Salmo gairdneri*) having a mean total length of 31 cm and a mean weight of 327 g, plus 19 brook trout (*Salvelinus fontinalis*) averaging 25 cm and 171 g, each with clinical whirling disease were selected from an affected population. Presence of the disease was confirmed by the most simple laboratory examination; in fact, every fish in a subsample had abundant spores. Yearling rainbow trout from Leetown National Fish Hatchery (West Virginia) stock were used as a source of uninfected skeletal elements.

About 100 L of soil from a fish hatchery pond and from an effluent basin containing vegetation and entrapped invertebrate fauna were obtained at West Virginia's Department of Natural Resources facility at Ridge, a hatchery with no known history of whirling disease. The materials were distributed at the rate of 20 L per tank (52 cm in diameter), which was then filled with spring water, free of *M. cerebralis*, to a depth of 36 cm. Water (see Warren 1963 for chemistry), was supplied at a rate of 600 mL per minute. Because of the presence of plant material, the tanks were kept under continuous fluorescent illumination to prevent oxygen depletion.

All whirling disease fish, a total of 33, were killed on the same day, eviscerated, bagged in plastic, and iced. The following morning, two-thirds of the fish were flown to the testing facility, placed in 8% brine solution (w/w) at the rate of 2 kg of brine per kilogram of fish, and held at 3.0 ± 0.8°C for 16 h. Fish were then removed from the brine, rinsed for 15 s in 0°C water, and blotted. Half (11 fish) were bagged and iced; the remaining 11 fish were further dried in a forced-air draft at 1°C for 1 h to help firm tissues before the smoking was begun. The process of smoking with hickory chips and sawdust began with a 100-min period of drying and further firming of flesh during which the temperature was gradually raised from 10 to 37.8°C. During the next 40 min the temperature was raised to 66°C and held for 40 min. Heat and smoke were then withdrawn. After being smoked, the 11 fish were cooled, bagged, and iced, and with the 11 brined fish flown back to the laboratory. Skeletal elements, principally those of the head, were removed, minced, and added to test aquatic environments the following day. One environmental unit was seeded with smoked fish material, another with brined fish, and a third with iced fish (to serve as a positive control). A fourth unit was seeded with tissues from healthy fish (the first negative control), and a fifth was kept as an environmental or second negative control to demonstrate that the aquatic soil itself harbored no *M. cerebralis*. Materials were allowed to age for 4 mo, before 60 susceptible rainbow trout fry were added. Eighty days later, after sufficient time for spore development, 55 fry from each unit were processed by the pepsin–trypsin–dextrose centrifugation method for spore release and concentration (Markiw and Wolf 1974).

The smoking operation was patterned after that described by Seagran et al. (1970) for compliance with the U.S. Food and Drug Administration's regulations governing the smoking of fish. The brined and air-dried trout were hung in the smoke chamber of an Afos brand forced draft mini-kiln (manufactured under work order 10415/8, Patent 1184363, Afos. Ltd., Anlaby, Hull, England[1]). Thermocouples from a Honeywell Elektronic 15 thermograph were placed in the tissues of five fish, in ambient air, in the atmosphere of the chamber, in a receptacle of water in the chamber, and in the stack. External charting permitted recording of temperature at critical points throughout the processing.

Results — Susceptible fry were held in the test environments for 80 d. At the end of that time the pepsin–trypsin–dextrose centrifugation method of spore release and concen-

[1]Mention of product name does not indicate Government endorsement of commercial product.

tration showed that viable spores were present only in the brined fish and in those of the positive control group, the group held continuously on ice. The test soil itself and the tissues from fish known to be free of *M. cerebralis* showed no evidence of infectivity.

Discussion — *Myxosoma cerebralis* spores were inactivated during the smoking process, but it is most likely that the heat and, more specifically, the peak temperature held for 40 min inactivated the spores. *Myxosoma cerebralis* survived brining that resulted in a mean NaCl content of tissue of 1.21% (range 0.95–1.57%), a value more than twice physiological levels. It seems logical, therefore, to attribute spore inactivation to temperature alone, i.e. the 40 min at 66°C. Hoffman and Markiw (1977) have shown that, for spores heated at 70°C for 40 min, an average of 82% are penetrated by methylene blue dye; moreover, 100% of the spores heated at 90°C for 10 min are dyed and presumed killed. Our results show that the lower temperature of 66°C for 40 min is lethal.

We conclude that hot smoking of trout infected with whirling disease offers a biologically safe way of preparing fish for human food that would otherwise be destroyed where eradication procedures are used as a control measure for whirling disease.

Acknowledgments — We thank H. Seagran, Director, Charleston Laboratory, National Marine Fisheries Service, Charleston, South Carolina, for permission to use the equipment, and personnel of his facility for our project; and Robert Ernst for carrying out the brining and smoking portions of this study and for determining the moisture and salt content of the final product.

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Substrate Preference Behavior of Juvenile American Lobsters, *Homarus americanus*, in Gravel and Silt–Clay Sediments

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POTTLE, R. A., AND R. W. ELNER. 1982. Substrate preference behavior of juvenile American lobsters, *Homarus americanus*, in gravel and silt–clay sediments. Can. J. Fish. Aquat. Sci. 39: 928–932.

In the laboratory, early stage juvenile American lobsters, *Homarus americanus*, showed a preference for spending time and selecting shelters in gravel when given a choice between silt–clay deposited over gravel versus gravel substrates. Lobsters excavated shelters in the gravel by various combinations of pushing, plowing, and carrying stones with their chelae, third maxillipeds, and walking legs. Burrows in silt–clay were invariably adjacent to supporting structures. Our observations on shelter modifications and occupancy indicate that juvenile lobsters are active primarily, but not exclusively, at night. The juvenile lobsters frequently changed shelters during the initial 3 d before a status quo was established. We hypothesize that ocean dumping of silt–clay over gravel may increase spatial competition among juvenile lobsters for shelter in remaining gravel habitat.

Key words: shelter, burrowing, competition, ocean dumping

POTTLE, R. A., AND R. W. ELNER. 1982. Substrate preference behavior of juvenile American lobsters, *Homarus americanus*, in gravel and silt–clay sediments. Can. J. Fish. Aquat. Sci. 39: 928–932.

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Printed in Canada (J6585)
Imprimé au Canada (J6585)

Quand on offre à de jeunes homards d'Amérique (*Homarus americanus*) en laboratoire un choix entre un substrat de vase−argile déposé sur du gravier et un de gravier seul, ils préfèrent ce dernier et y passent plus de temps. Pour se creuser des abris, ils combinent de diverses manières le poussage, le charruage et le transport des cailloux avec leurs pinces, troisièmes maxillipèdes et péréiopodes. Les tunnels qu'ils creusent dans la vase−argile sont toujours situés à proximité de structures de support. Nos observations sur les modifications apportées à ces abris, ainsi que sur le temps qu'ils y passent, indiquent que les juvéniles sont actifs surtout la nuit, bien que non exclusivement. Dans les trois premiers jours, il y a changements fréquents d'abris tant qu'un statu quo n'a pas été établi. Nous avançons l'hypothèse que le déversement en mer de vase−argile sur le gravier pourrait résulter en une concurrence accrue pour les abris parmi les jeunes homards dans l'habitat graveleux qui reste.

Received August 11, 1981
Accepted March 8, 1982

Reçu le 11 août 1981
Accepté le 8 mars 1982

ALTHOUGH the American lobster, *Homarus americanus*, is of major commercial importance along the east coast of Canada and the USA, the ecology and behavior of the early juvenile stages are poorly known. As these stages are difficult to locate and observe on the seabed, laboratory investigations into their shelter-related behavior may help elucidate their natural substrate requirements and distribution.

Stage IV larvae of *H. americanus* usually prefer a gravel substrate to one of sand or mud and may delay molting to the benthic fifth stage until "favorable (rocky) conditions are reached" (Cobb 1968). However, comparable data on the substrate preferences of subsequent juvenile stages are lacking. In the present laboratory study, we tested the preference of *H. americanus* juveniles (stages VII−X) for spending time and seeking shelter in either a shallow layer of silt−clay sediment deposited over gravel or in gravel. Additionally, we recorded the behavior involved in the choice and excavation of shelters.

Materials and methods — We obtained thirty-two juvenile *H. americanus* of 9- to 15-mm carapace length (CL) (~stages, VII−X) from a hatchery at the Department of Fisheries and Oceans Biological Station at St. Andrews, New Brunswick. Prior to testing they were held in individual compartments in running seawater at temperatures of 7−10°C and were fed crushed *Mytilus edulis* or finely chopped *Illex* sp. twice weekly.

Tests were conducted in two 0.9 × 0.9 m fiberglass tanks filled with seawater to a depth of 0.2 m. Each tank was subdivided into four 0.45-m square compartments with 0.1 m high perforated Plexiglas partitions. The tank bottom was covered by ~30 mm of unsorted gravel from the surface of the intertidal zone at Brandy Cove, St. Andrews. Mean particle size was ~20 mm. The material also included stones ≤80 mm diameter, shell fragments, and coarse sand.

We collected mixed silt−clay sediment containing ~30% sand from the surface of the intertidal zone at Pottery Creek, 2 km east of Brandy Cove. The sorting characteristics and organic carbon content of the silt−clay sediment, described by Wildish et al. (1977), are typical of dredge spoil in the region. The sediment was deposited over one half of the gravel surface in each tank compartment and varied in depth from <10 mm at the silt−clay/gravel border to ≤40 mm at the center of the deposit, which covered a 0.23-m × 0.45-m area. Large stones protruded from the sediment in some tank compartments but by no more than 10 mm. We siphoned off silt−clay which settled in the "clear" areas during deposition.

To avoid sediment disturbance there was no inflow of seawater during the 5- to 9-d trials. Water temperature was initially adjusted to holding tank temperature and ranged from 10.5 to 13.0°C. Air stones suspended just below the surface provided aeration, and illumination was by natural light. All trials were conducted between January 14 and March 6, 1980.

We placed individual juvenile lobsters in separate tank compartments between 10:00 and 16:00. During placement, each lobster was confined within a 25-mm-diameter plastic pipe placed vertically on the gravel/silt−clay intersurface. The pipe was slowly lifted after 10 min, exposing the lobster. We plotted locomotor activity on a diagram of the tank compartment for 15 min and marked the location of the lobster on the plot at 30-s intervals to provide an approximate record of the time spent on each substrate. We also recorded qualitative data on initial burrowing and exploratory behavior. Lobsters which attempted to climb the compartment partitions were gently dissuaded with a thin rod.

After the initial observations, we recorded the positions of the four lobsters in each of the two tanks at 08:30 and 16:00 for 5−9 d and noted the construction of new burrows or changes of shelter location. Location of concealed animals often required some minor disturbance of the gravel substrate, but we took care to replace material in its original configuration. If we could not locate the missing lobster without major disturbance of the substrate, we abandoned the search. Tunnels in silt−clay were not probed. We replaced the water in the test tanks and filled all excavations in gravel and silt−clay prior to the introduction of new animals.

Results and discussion — We considered a lobster to have selected a shelter site when it was either wholly or partly concealed in the substrate and immobile, or engaged in shelter excavation, by the end of the initial observations. Twenty-nine (90.6%) of the animals selected a shelter within 15 min of introduction to the tank ($\bar{x}$ = 4.7 min, range: 0.5−14.0 min). Significantly more of these (24) selected a shelter site in gravel than in silt−clay (χ^2 test, $P < 0.01$). Four juveniles, which selected shelter sites in silt−clay, began an excavation adjacent to a protruding stone: in one case, in an existing excavation which had been incompletely filled between trials. One other lobster excavated a burrow in silt−clay by a tank corner.

We converted time spent on each substrate to proportion of total time. The mean time spent on or within gravel during the initial 15-min observations was 11.4 min or 0.76 of total time

TABLE 1. Mean proportion of time spent on or within gravel substrate (Tg) by juvenile *H. americanus* during initial 15-min observations; for lobsters exceeding 0.50 of time on gravel (+) and lobsters spending 0.50 or less of time on gravel (−).

Sign	Tg	±SD	Range	*n*
+	0.90	±0.12	0.60−0.99	25
−	0.23	±0.17	0.07−0.50	7

($n = 32$). Trials in which the proportion of total time on gravel exceeded 0.50 were assigned a positive value, whereas those in which the proportion was 0.50 or less were considered negative (Table 1). The normal approximation to the binomial distribution revealed that the proportion of trials in which more than 0.50 of total time was spent on gravel ($P = 25/32 = 0.78$) was significantly higher than that expected if there was no preference between substrates (*t*-test, $P < 0.01$).

Lobsters tended to remain close to the compartment walls during initial exploration of the tank. They ventured away from the walls more frequently on gravel than on silt−clay and traversed gravel areas more slowly, primarily because of frequent probing of crevices with the chelipeds. One or both chelipeds were used in probing, depending upon the size of the interstitial space. If both chelipeds were used, they were extended forward, with chelae closed and in contact for most of their length, as the lobster advanced into the opening. The inserted chelae were spread until contact was made with the edges of the crevice. Small juveniles usually obtained shelter before large individuals, probably because there were more crevices which could accommodate them without enlargement.

Lobsters of <10 mm CL were able to shift stones ≤25 mm in diameter by moving beneath an overhanging edge and pushing upward and/or sideways with the walking legs. Stones of <10 mm diameter were removed from excavations in gravel by: (1) plowing with the third maxillipeds and the first one or two pairs of walking legs; (2) grasping and carrying with one or both chelae; (3) pushing with both chelae; or (4) more rarely, carrying a stone between both closed chelipeds. The third maxillipeds were used to remove coarse sand particles. Burrowing behavior in the deposited silt−clay was similar to that described for lobsters by Cobb (1971) and Berrill and Stewart (1973). Burrows in silt−clay generally extended through the deposited silt−clay layer and into the underlying gravel substrate.

Seventy-one postintroduction observations were made on the eight groups of four lobsters per tank. We found that burrowing activity was infrequent during daylight hours, and that all construction of new burrows and major modification of existing burrows occurred overnight. Burrows in silt−clay were conspicuous and large excavations in gravel were marked by debris deposited in front of the entrance(s) (Fig. 1A, B). However, the locations of lobsters concealed in unmodified crevices were difficult to ascertain, unless the antennules or tips of the chelae were exposed. The mean number of animals located per observation per tank was 3.4 (85.0%). In 242 sightings of lobsters in shelters, 194 animals (80.2%) were in gravel. This number is significantly higher than the expected frequency (121), based on the assumption that there is no preference of gravel over silt−clay for shelter location (χ^2 test, $P < 0.005$).

Lobsters frequently changed location between observations and some individuals had two or three shelters. Even the most extensive excavations in gravel were occasionally vacant. Figure 1B shows two shelters utilized by the same juvenile. Both were in use during the same period, although the excavation beneath the large stone on the gravel/silt−clay interface had collapsed by the time the photograph was taken. The Plexiglas partitions were not effective in separating the animals, and as many as three juveniles were present in a single tank compartment, with burrow entrances as close as 0.1 m. Shelter occupants changed on several occasions in the initial 3 d before a status quo was established.

Depending on whether nonlocated individuals in previous observations had or had not moved, a mean of either 0.67 or 1.13 animals ($n = 24$) moved between consecutive daylight observations (at 08:30 and 16:00) and a mean of either 1.30 or 1.68 animals ($n = 47$) moving overnight. A number of moves may have been caused by disturbance of the substrate during the searches for concealed lobsters. Undisturbed lobsters changed location less frequently during daylight than overnight and were seldom seen outside of their shelters before dark. These observations and others on extensive nocturnal modifications to shelters indicate that juvenile *H. americanus* are active primarily, but not exclusively, at night.

The strong initial preference for gravel over silt−clay by *H. americanus* juveniles might have been due, in part, to their introduction to the apparatus under conditions of full illumination. Howard and Bennett (1979) found that, although *Homarus gammarus* juveniles tested in light strongly preferred stones to unsieved cohesive mud, animals in darkness exhibited wider habitat tolerance. However, an overall preference for shelter in gravel substrate persisted throughout our 5−9 d of daylight observations on *H. americanus*.

We believe that *H. americanus* juveniles chose gravel substrate over silt−clay because the latter lacks readily available shelter, and because evacuation and maintenance of burrows in silt−clay may be difficult in the absence of supporting structures such as embedded stones. The known presence of *H. americanus* juveniles in the gravel and boulder substrates of inshore areas (R. W. Welsford, Research Group Limited, Historic Properties, 1869 Upper Water Street, Halifax, N.S. B3J 1S9, personal communication) warrants concern regarding possible detrimental effects of ocean dumping on lobster "nursery areas." Assuming that juvenile lobsters prefer gravel areas in the field, smothering of gravel substrate, due to dumping of dredge spoils or settling out of suspended materials, may result in increased spatial competition among lobsters for shelter in remaining gravel habitat. Scarratt (1968) monitored the colonization of an artificial reef and suggested that the scarcity of suitable cover may limit lobster populations. Adverse results of spatial competition could include increased lobster mortality from predation, caused by a decreased availability of suitable shelters and subsequent increased vulnerability to predators. In addition, lobsters could exhibit a decreased growth rate from crowding, due to spatial competition, as observed in the laboratory by Cobb and Tamm (1974) and Stewart and Squires (1968).

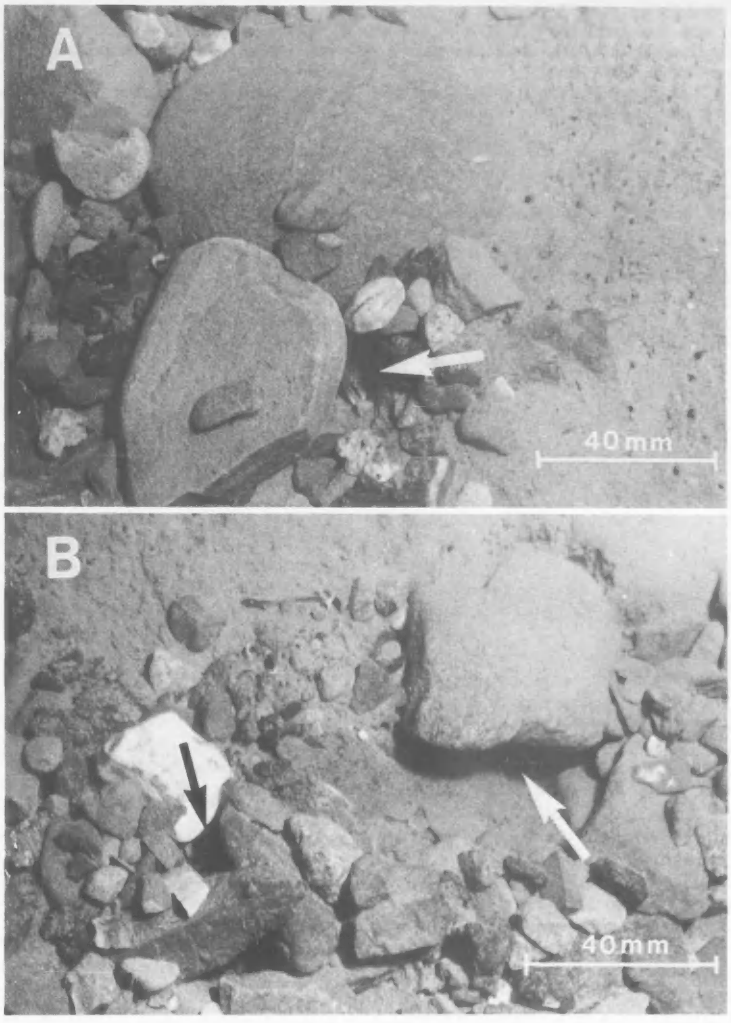

FIG. 1A. Juvenile *H. americanus* burrow under stone adjacent to gravel/silt–clay interface. Note excavated material and protruding antennule and chela. B. Two shelters utilized by a single juvenile *H. americanus*. Note excavated stones and sediment to left of hollow beneath large rock and small stones removed from the shelter in gravel adjacent to the white stone.

Because juvenile American lobsters may be similar to their European counterparts (*H. gammarus*) in that they inhabit substrates that are different from their adult conspecifics (Howard 1980), dump site selection in the Canadian maritime provinces, generally harbor dredgings, should not be based solely on the absence of commercial size lobsters. The dredging or smothering of habitats occupied by juvenile lobsters ("nursery" areas) could be a serious threat to the local lobster fishery.

Acknowledgments — We thank Drs A. Campbell, R. H. Peterson, J. D. Pringle, and Mr J. F. McElman for their constructive criticism of earlier drafts of the manuscript. Dr D. E. Aiken provided the juvenile lobsters. The work was funded in part by the Regional Ocean Dumping Advisory Committee.

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BOOK REVIEWS/COMPTES RENDUS

Salmonid Ecosystems of the North Pacific. *Edited by William J. McNeil and Daniel C. Himsworth. Oregon State University Press, Corvalis, Oregon. 1980. 331 p. $15 U.S.*

This book is a collection of papers presented at a 1978 symposium. It is a useful addition to the literature because it summarizes available information on the biology of Pacific salmon. Perhaps the title would lead a casual observer to expect it to deal exclusively with issues related to salmon in the North Pacific Ocean; however, the title does not adequately reflect its contents. A diverse array of papers deal with freshwater, and estuarine and oceanic environments and cover such topics as trends in abundance, density dependence in the marine environment, early marine life history, population genetics, and a group of papers which defy easy categorization. Some of this latter group and the abstracts submitted by seven Soviet scientists hardly seem relevant to the major theme of the book which relates to the capacity of the marine environment to produce more salmon.

The book serves the important function of bringing together past work, much of which is hidden away in relatively obscure government documents, into a form which is more generally available. Its utility would have been increased by arranging the papers into logical groups and by providing abstracts for each paper. It would also have been helpful had the editors summarized the general thrusts emerging from these papers and perhaps pointed out data deficiencies and research needs.

One group of papers deals with changes in abundance of salmon and illustrates the general decline of some species over the last 50 or more years. Collectively they indicate the importance of enhancement in the maintenance of other stocks. The potential value of enhancement and the problems of stock management come through loudly and clearly.

Two papers in a second group deal with the significance of density dependence in the marine environment. These are important contributions in an area that has received too little attention. Perhaps the results were not always conclusive but these papers, and some more recently published, strongly suggest that density dependence is a factor affecting size and abundance of adult salmon. Enhancement and stock management strategies should be developed with these types of data in mind.

The papers in this volume that deal with the early marine life history of juvenile salmonids and salmon population genetics are significant contributions containing considerable new information. The papers by Healey and Utter et al. are particularly noteworthy and are amongst the best in this book. The six papers in the genetics and life history groups are of considerable importance given current concerns for the effects of enhancement programs. More work is badly needed on both salmon population genetics and the early marine life history of young salmon.

Several papers do not easily fit into any category. Some, important in their own right, are of limited value in this volume. The paper on the role of the pineal gland in migration is one such example. The value of the paper on North Pacific primary productivity would have been increased if the author or editors had attempted to bridge the gap between phytoplankton and salmon by indicating something of our knowledge of planktonic animals in the North Pacific Ocean.

Is this book worth having on your bookshelf? The answer is, on the whole, positive. It represents a useful summary of available information, not all of which is easy to obtain for students or practicing biologists. Some of the material reviewed in several papers is more than a decade old (such as that on high seas salmon distributions), but is the best we have and not likely to be improved on in the near future given current constraints on fisheries research. Despite its limitations, the book is good value in this time of high book prices.

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(R-332)

Marine Botany. *Clinton J. Dawes. John Wiley & Sons, Toronto & New York. 1981. ix + 628 p. $45. (U.S.)*

Although *Marine Botany* is not the phycology book we have all been waiting for, it certainly fills a gap in the literature and provides a different perspective than present texts. In presenting an overview of marine botany from an ecological viewpoint, we should all be grateful to Professor Dawes. The book is divided into five sections: 1, an introduction to the plants, their environment and uses; 2, an overview of the taxonomy and morphology of algae; 3, a description of the marine environment and ecological methods; 4, a discussion of the principal plant communities; and 5, appendices covering the taxonomy and biology of marine fungi, lichens and bacteria. *Marine Botany* is not a monograph of the field, but a synthesis to be used as an advanced textbook or general reference work.

The strengths of the book lie in the ecological sections. Chapters 10–14 include considerable information on ecological methods with clear-cut directions on a variety of field and laboratory procedures. This is well integrated into discussions of theory and application of the methods and culminates in what is possibly the most useful chapter (14) on physiological ecology. Less pure description than older texts, the reader gets the impression of the main plant ecosystems as functioning entities (chapters 15–20). Thus we are presented with information on adaptations to particular environments, and the various processes of competition and herbivory. The experimental ecology of Lubchenco, Paine, and others is discussed and there are numerous recent references. The chapter on uses of algae is an excellent summary, although missing is information on kelp farming in China.

Despite these positive aspects to the book, there are a number of problems that limit its effectiveness. Throughout

the book there is a general tendency toward a superficial treatment of material without adequate critical assessment or discussion of opposing viewpoints. This is unfortunate in what is billed as an advanced text. Although the book is well produced with numerous drawings, photographs, and tables, it is fraught with misspellings and typographical errors; these being especially disturbing in taxonomic names. The red algal chapter alone contained 15 names with errors — the most obvious being "*Galaxura*." On the lighter side, a *Porphyra* is described as being "monstromatic," and one figure legend refers to two cells when four cells are shown.

The weakest section of *Marine Botany* is the morphology and taxonomy (chapters 4–9). After a brief overview of each algal division, the classes, and selected orders, families, and genera are discussed. This material has all been reviewed in greater detail and with greater authority in other recent texts. For many taxa I find the treatment is either simplistic or discusses aspects of the biology that may be out of date, or represent only one side of controversial subjects. A number of misleading or incorrect statements are made about various organisms, and there are some inconsistencies from one part of the text to another. Dawes states that there are "only about 3 freshwater" members of red algae (p. 173) when there are at least 20 genera. He also tells us that most Euglenophyceae are photosynthetic (p. 267), when the reverse is true. *Ulonema* is referred to as a green rather than a brown alga, and 'maerl' is first discussed (p. 36) as being composed of *Lithothamnium* and *Phymatolithon*, whereas later (p. 179) 'marl' is described as a product of *Corallina*.

One important subject that is virtually overlooked is the topic of life histories in an ecological context. There is no reference to algal life forms, or little discussion of algal seasonality, i.e. phenology, periodicity, and reproductive ecology. Thus what is potentially a unifying theme for phycology as a whole (and could have been a good focus for the taxonomic chapters) is not covered. As a result, the reader gets a strong idea of the importance of climatic, physical, and chemical influences in determining plant occurrence, but the relationships between the seasons and plant development, reproduction, and senescence are omitted.

For all its faults, marine biologists should find in *Marine Botany* a general reference work on algal ecology, and for the phycologist it complements the taxonomy and morphology of Bold and Wynne,[1] and will be used as a major source of information in teaching.

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(R397)

[1] *Introduction to the Algae*, Prentice-Hall, 1978.

Books Received/Livres reçus[1]

March/mars 1982

Journal of Freshwater Ecology. *Oikos Publishers, Inc., Box 2558, La Crosse, Wisconsin 54601. 1981 — Volume 1 (Numbers 1, 2, and 3). 340 p. Subscriptions: library/institutional, $35 per volume; individuals, $25 per volume.* A single volume will normally consist of six issues and will be published each year (the first volume of the journal will continue into 1982). This new journal is intended to be a vehicle for the reasonably rapid dissemination of current limnological information. Authors are able to revise their manuscripts in light of referee comments after acceptance and before printing, which is from authors' camera-ready copy. [R421]

The Limits and Relationships of the Lutjanidae and Associated Families. *G. David Johnson. Bulletin of the Scripps Institution of Oceanography, Volume 24. University of California Press, Berkeley and Los Angeles, California, and University of California Press, Ltd., London, England. 1981. 114 p. $8.* This study attempts to provide the anatomical information necessary for the elucidation of the previously confusing array of families associated with the Lutjanidae. In addition to osteological characters, certain muscle complexes are considered. A classification to the generic level is proposed. [R418]

Contributions to a Guide to the Cypriniform Fish Larvae of the Upper Colorado River System in Colorado. *Darrel E. Snyder. Biological Sciences Series Number 3. United States Bureau of Land Management, Colorado. 1981. vi + 81 p.* This publication is intended to make prepared information on larval fish identification and the cypriniform larvae of the Upper Colorado River System available to potential users. Additional data are yet to be summarized. Ultimately a relatively comprehensive guide, which will include most of the text presented herein, keys by developmental phase, and a species account, will be possible. Although the current document is designed primarily for use in the southwestern United States, many species are found in other waters. [R419]

Salmon Ranching. *Edited by J. E. Thorpe. Academic Press, New York. 1980 (Publication date 1981). 441 p. $78 (U.S.).* The contributors to this book have shown that the artificial propagation of salmon has grown from a compensatory activity to environmental modification into a world-wide exploitative industry. The book presents the most comprehensive review to date of salmon ranching throughout the world. [R420]

[1]Persons interested in reviewing books in this list should contact the Book Review Editor, Canadian Journal of Fisheries and Aquatic Sciences, Scientific Information and Publications Branch, Department of Fisheries and Oceans, Canada K1A 0E6.

[1]Les personnes intéressées à examiner les livres énumérés dans cette liste sont priées de communiquer avec le Rédacteur des comptes rendus, Journal canadien des sciences halieutiques et aquatiques, Direction de l'information et des publications scientifiques, Ottawa, Canada, K1A 0E6.

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Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

CJFSDX39(6) 811–936 (1982)
ISSN 0706-652X

Volume 39, No. 6, June 1982

Volume 39, n° 6, juin 1982

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